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(71) Applicant *(for all designated States except US)*: **AGRESEARCH LIMITED** [NZ/NZ]; Ruakura Campus, East Street, Hamilton (NZ).

(71) Applicants and
(72) Inventors: **LAITINEN, Mika, Petri, Esaias** [FI/FI]; Tarkk ampujankatu 14 B 46, FIN-00150 Helsinki (FI). **RITVOS, Olli, Visa-Pekka** [FI/FI]; Visakoivuntie 10 G 13, FIN-02130 Espoo (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **DAVIS, George, Henry** [NZ/NZ]; Invermay Agricultural Centre, Private Bag 50034, Mosgiel, Dunedin (NZ). **GALLOWAY, Susan, May** [NZ/NZ]; AgResearch Molecular Biology Unit, Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin (NZ). **JUENGEL, Jenny** [US/US]; Wallaceville Animal Research Centre, P.O. Box 40063, Upper Hutt (NZ). **MCNATTY, Kenneth, Pattrick** [NZ/NZ]; Wallaceville Animal Research Centre, P.O. Box 40063, Upper Hutt (NZ). **VUOJOLAINEN, Kaisa, Niina, Johanna** [FI/FI]; Koskelantie 23 H 67, FIN-00610 Helsinki (FI).

(74) Agents: **WILSON, Kathryn, S.** et al.; KPMG Centre, Level 12, 85 Alexandra Street, Private Bag 3140, Hamilton (NZ).

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(54) Title: NUCLEOTIDE AND AMINO ACID SEQUENCES OF OOCYTE FACTORS FOR ALTERING OVARIAN FOLLICULAR GROWTH IN VIVO OR IN VITRO

(57) Abstract: The present invention relates to nucleotide and amino acid sequences of oocyte factors for altering ovarian follicular growth *in vivo* or *in vitro*. The present invention also concerns novel homodimeric and heterodimeric polypeptides and their use for altering mammalian ovarian follicular growth *in vivo* or *in vitro*. In particular, the invention broadly concerns active or passive immunisation against these homo- or heterodimeric polypeptides or functional fragments or variants thereof so as to alter follicular growth *in vivo* or *in vitro*.

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NUCLEOTIDE AND AMINO ACID SEQUENCES OF OOCYTE FACTORS FOR ALTERING OVARIAN FOLLICULAR GROWTH *IN VIVO* OR *IN VITRO*

The present invention relates to nucleotide and amino acid sequences of oocyte factors for altering ovarian follicular growth *in vivo* or *in vitro*. The present invention also concerns novel homodimeric and heterodimeric polypeptides and their use for altering mammalian ovarian follicular growth *in vivo* or *in vitro*. In particular, the invention broadly concerns active or passive immunisation against these homo- or heterodimeric polypeptides or functional fragments or variants thereof so as to alter follicular growth *in vivo* or *in vitro*.

BACKGROUND OF THE INVENTION

The molecular nature of regulatory molecules responsible for stimulating early phases of ovarian folliculogenesis (i.e. the growth and differentiation of primordial follicles to primary, secondary, and pre-antral follicles) has been poorly understood. On the other hand, follicle-stimulating hormone (FSH) and luteinising hormone are glycoprotein hormones derived from the pituitary and have generally been accepted as the key factors regulating the later stages of ovarian folliculogenesis. Moreover FSH is accepted as the single most important factor for stimulating a greater than normal number of follicles to ovulate, a fact that is well illustrated by the wide use of commercial FSH preparations in ovarian hyperstimulation regimes both in medicine and veterinary medicine. Recent studies have indicated that early folliculogenesis is controlled by intraovarian factors of which the granulosa cell-derived stem cell factor (or c-kit ligand) and the oocyte-derived growth differentiation factor-9 (GDF-9) have gained most attention, because both appear to be essential for early mammalian folliculogenesis.

GDF-9 was first described in 1993 as a novel member of the transforming growth factor beta (TGF-β) superfamily which is specifically expressed in the ovary (McPherron and Lee, 1993). Like other members of the TGF-β family, GDF-9 is encoded as a prepropeptide consisting of a signal peptide, a proregion, and a so called C-terminal mature region, which is cleaved from the precursor peptide by an intracellular protease belonging to a group of furin-like proteases. Growth factors of the TGF-β family are characterised by a common pattern of cysteine residues found in the mature region that is likely to form in all members of the family; this is a rigid intramolecular structure known as the

"cysteine knot" which is composed of six Cys residues forming three characteristic disulphide bridges in a monomer of a TGF-β family member (Daopin et al., 1992; Schlunegger and Grutter, 1992 and 1993; Griffith et al., 1996; Scheufler et al., 1999). Most members of the TGF-β family have a conserved seventh Cys residue that is responsible for the covalent homodimerisation of two identical monomers (homodimers) or for the heterodimerisation of a given TGF-β family member with another distinct member of the family (heterodimers).

In mice, GDF-9 is expressed in oocytes from the primary stage of follicular development until ovulation (McGrath et al., 1995; Laitinen et al., 1998). Using the mouse GDF-9 sequence as a test sequence for the data base searches we identified, a GDF-9 like expressed sequence tag (EST) cDNA derived from a 2-cell mouse embryo library (Laitinen et al., 1998). We showed that the transcript of this novel factor, GDF-9B, which is 55% homologous to GDF-9, is expressed in oocytes of the mouse ovary at the same time as GDF-9 (Laitinen et al., 1998). Using PCR and primers derived from the mouse EST sequence we amplified a fragment of the corresponding gene from human genomic DNA, mapped the gene locus to chromosome Xp11.2, and deduced the human GDF-9B gene structure from isolated cosmid clones (Aaltonen et al., 1999). Interestingly, in the human ovary, GDF-9 mRNA expression begins in primary follicles slightly earlier than that of GDF-9B (Aaltonen et al., 1999). The mouse and human GDF-9B genes have been cloned and the protein encoded by the gene has also been named bone morphogenetic protein 15 (BMP-15) (Dube et al., 1998).

GDF-9 appears to be essential for ovarian folliculogenesis. From the literature it is known that GDF-9 deficient mice (GDF-9 -/-) are infertile due to an early arrest in folliculogenesis (Dong et al., 1996). In GDF-9 -/- ovaries folliculogenesis stops at the primary follicle stage when one layer of cuboidal granulosa cells surrounds the oocyte. Even though the oocyte continues to grow, the granulosa cells fail to proliferate and no thecal cell differentiation is associated with follicular enlargement.

The Inverdale fecundity gene ($FecX^I$) was identified as a major gene affecting the prolificacy of a Romney flock of sheep (Davis et al., 1991). Segregation analyses determined that the gene is carried on the X-chromosome, and that ewes carrying a single copy of the gene (I/+) have litter size about 0.6 lambs larger than noncarrier ewes (+/+). The increase in number of lambs born is directly linked to an altered pattern of follicular development and an increase in ovulation-rate above that in the

wild-type of ~1.0 (Shackell et al., 1993; Davis et al., 1991). By contrast, homozygous carrier ewes having two copies of the gene (I/I) are infertile; due to an ovarian failure condition (Davis et al., 1992). In ovaries of the (I/I) ewe, folliculogenesis stops at the primary follicular stage and the phenotype is not dissimilar to that seen in GDF-9 (-/-) mice (Braw-Tal et al., 1993; McNatty et al., 1995; Smith et al., 1997).

A second prolific Romney flock (Hanna, 1995) with no known connection to the Inverdale flock was also shown to carry an X-linked mutation with similar phenotype to Inverdale. Evidence that the Hanna animals carried a mutation ($FecX^{H}$) in the same gene as for Inverdale was obtained when infertile females were produced by mating Inverdale carrier rams with carrier Hanna ewes (Davis et al., 1995). The Hanna line has been maintained at the Invermay AgResearch Centre as a distinct group alongside the Inverdale line.

In New Zealand Patent Application No. 500844 we, the present inventors, identified in Inverdale sheep a nucleotide substitution beyond the mature peptide processing site of the GDF-9B gene which converts the codon GTC (amino acid valine (V) to GAC (amino acid aspartic acid (D)). We also showed that in Hanna sheep the C nucleotide beyond the mature peptide processing site is converted to a T. This converts a codon CAG (coding for glutamine (Q)) to a codon TAG (coding for termination) thereby resulting in a truncated mature protein. These respective mutations in Inverdale and Hanna are suggested to be the underlying causes for the "streak" ovaries and anovulatory conditions in homozygous Inverdale or Hanna ewes and in the Inverdale cross Hanna ewes.

Previously it has been shown that GDF-9 -/- mice are infertile showing that GDF-9 is important for normal fertility in some mammals. However, with the discovery of the related oocyte-specific factor GDF-9B several novel findings were found by us to support the notion that: (1) GDF-9B is essential for normal folliculogenesis in some mammals; (2) that GDF-9B is critically important for determining the ovulation rate in some mammals and; (3) since GDF-9 and GDF-9B are co-expressed by oocytes, they function co-operatively to enhance both follicular development and ovulation rate. Collectively these novel hypotheses were only made possible by our discovery of the Inverdale and Hanna GDF-9B mutations in sheep.

The inventors have for the first time determined the full gene structure of the sheep GDF-9B gene encoding the wild-type protein and have shown that it is necessary for maintaining normal ovarian folliculogenesis in sheep. The inventors have also identified the full gene structure of the GDF-9B variants in Inverdale and Hanna sheep which cause higher than normal ovulation rates in heterozygous animals and infertility in homozygous animals. It is broadly to the full-length wildtype and mutated GDF-9B sequences and variants thereof and their use in the modulation of mammalian fertility that the present invention is directed.

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in New Zealand or in any other country.

SUMMARY OF THE INVENTION

Accordingly, to a first aspect, the present invention provides an isolated wildtype GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) SEQ ID No:1;
b) a sequence able to hybridise under stringent conditions to the molecule in (a);
c) a sequence which is a functional variant or fragment of the molecule in (a);
d) a sequence complementary to the molecule defined in (a), (b) or (c); and
e) an anti-sense sequence corresponding to any of the molecules in (a) – (d).

In a second aspect, the present invention provides an isolated full length mutated GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

a) SEQ ID NO: 3 or SEQ ID NO: 5 ;
b) a sequence able to hybridise under stringent conditions to the molecule(s) in (a);
c) a sequence which is a functional variant or fragment of the molecule(s) in (a);
d) a sequence complementary to the molecule(s) defined in (a), (b) or (c); and
e) an anti-sense sequence corresponding to any of the molecule(s) in (a) – (d).

The nucleic acid molecule may be an RNA, cRNA, genomic DNA or cDNA molecule, and may be single- or double-stranded. The nucleic acid molecule may also optionally comprise one or more synthetic, non-natural or altered nucleotide bases, or combinations thereof.

In a third aspect, the present invention provides an isolated full-length GDF-9B polypeptide comprising an amino acid sequence selected from the group consisting of:

a) SEQ ID NO:2 , SEQ ID NO:4 ; or SEQ ID NO:6; and
b) A functional variant or fragment of the sequence(s) in (a).

In a fourth aspect, the present invention provides a homodimeric mature GDF-9B polypeptide having subunits comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2 or a functional fragment or variant of said sequence.

In the fifth aspect, the present invention provides a heterodimeric polypeptide having subunits selected from the group consisting of:

a) A mature GDF-9B polypeptide comprising an amino acid sequence derived from SEQ ID NO:2 or functional fragment or variant of said sequence; and
b) A mature GDF-9 polypeptide or a functional variants or fragments thereof.

The present invention further provides in a sixth aspect a method of expressing biologically active processed homodimeric GDF-9B polypeptide comprising the steps of:

a) generating an expression construct comprising a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 or a functional fragment or variant of said sequence of the group;
b) transfecting a suitable cells with said construct;
c) selecting stable clones; and
d) isolating and purifying the expressed polypeptide.

In a seventh aspect, the present invention provides a method of expressing biologically-active processed heterodimeric GDF-9B and GDF-9 polypeptides comprising the steps of:

a) generating an expression construct containing a nucleic acid molecule comprising:
   (i) a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1 or a functional fragment or variant of said sequence; and
   (ii) a nucleic acid molecule encoding GDF-9 or a functional fragment or variant thereof;
b) transfecting suitable cells with said construct;
c) selecting stable clones; and isolating and purifying the expressed polypeptide

Preferably the cells transfected are vertebrate, however the use of other cell types is envisaged.

The GDF-9 nucleic acid and protein sequences are available in public databases such as GENbank and SWISS-PROT. The accession number for the sheep GDF-9 nucleic acid is AFO78545 and for protein is AAC28089.

Also provided by the present invention are recombinant expression vectors which contain a DNA molecule of the invention or functional variant thereof, and hosts transformed with a vector of the invention capable of expressing a polypeptide of the invention.

An additional aspect of the present invention provides a ligand which binds to a polypeptide of the invention. Most usually, the ligand is an antibody. It should be appreciated that the term "antibody" encompasses fragments or analogues of antibodies which retain the ability to bind to a polypeptide of the invention, including but not limited to Fv, $F(ab)_2$ fragments, ScFv molecules and the like. The antibody may be polyclonal or monoclonal, but is preferably monoclonal. In some embodiments the ligand may be a phage display molecule generated against polypeptides of the present invention, a single cell surface receptor or complex cell surface receptor. The polypeptide or peptide may be present as a monomer, dimer, heterodimer, multimer or a variant thereof.

In an eighth aspect, the invention provides a method for assessing the activity of GDF-9B homodimers and/or GDF-9B/GDF-9 heterodimers, comprising the steps of:

a) adding an effective amount of a GDF-9B homodimeric polypeptide; and/or a GDF-9B/GDF-9 heterodimeric polypeptide to an ovarian cell or organ culture with or without other ovarian growth factors such as IGF-1 and/or other members of the transforming growth factor superfamily (e.g. activin, BMP2, TGFβ1); and

b) conducting a bioassay on said cell or organ culture to assess the biological activity of said homodimeric and heterodimeric polypeptides.

In a ninth aspect, the invention provides transgenic animal models useful for demonstrating the effect of systemic production of GDF-9B homodimers and GDF-9B/GDF-9 heterodimers on follicular growth.

In a tenth aspect, the invention provides a method of adenoviral, retroviral and alphaviral transfer of GDF-9B expression cassettes or GDF-9 expression cassettes to host cells or organisms to thereby effect *in vivo* expression of GDF-9B homodimers or GDF-9B/GDF-9 heterodimers, comprising the step of transferring into a recipient cell, organ culture or recipient animal, a recombinant adenovirus including an expression cassette comprising a nucleic acid molecule having a nucleotide sequence selected from the group consisting of SEQ ID NO:1 or a functional fragment or variant of said sequence, said nucleic acid molecule being in operative association with an expression control sequence.

In an eleventh aspect the present invention provides the use of an agent selected from the group consisting of:

a) a homodimeric polypeptide having subunits comprising GDF-9B or a functional fragment or variant thereof with or without homodimeric polypeptide having subunits comprising GDF-9 polypeptide or a functional fragment or variant thereof;
b) a heterodimeric polypeptide having subunits comprising GDF-9B and GDF-9 polypeptides, or functional fragments or variants of said GDF-9B or GDF-9 polypeptides;

together with or without supplementary gonadotrophins (e.g. FSH and/or LH) and/or other ovarian growth factors such as IGF-1, kit ligand (stem cell factor), epidermal growth factor or a member of the TGFβ superfamily (i.e. an agonist or antagonist) to:

1)alter follicular growth in ovaries of a mammal or other vertebrate either *in vivo* or *in vitro;*

or

2) alter isolated ovarian cell growth/maturation *in vitro* (e.g. oocyte – cumulus cells and/or granulosa cells)

In yet a twelfth aspect, the invention provides a composition comprising an effective amount of an agent selected from the group consisting of:

a) a homodimeric polypeptide having subunits comprising a GDF-9B polypeptide or a functional fragment or variant thereof with or without homodimeric polypeptide having subunits comprising GDF-9 polypeptide or a functional fragment or variant thereof;

b) a heterodimeric polypeptide having subunits comprising a GDF-9B polypeptide and a GDF-9 polypeptide, or functional fragments or variants of said GDF-9B or GDF-9 polypeptides;

together with a pharmaceutically or veterinarily acceptable carrier (including adjuvants) or diluent; and optionally including supplementary gonadotrophins and/or other relevant ovarian growth factor agonists/antagonists.

In a thirteenth aspect the invention provides a method of altering ovarian follicular growth in a female mammal or other female vertebrate, *in vivo* said method comprising the step of transforming mammalian and other vertebrate ovarian host cells with GDF-9B and GDF-9 expression cassettes to allow over-expression of GDF-9B homodimers and GDF-9B/GDF-9 heterodimers.

In a fourteenth aspect the invention provides a method of altering ovarian follicular growth in a female mammal or other female vertebrate, *in vitro* said method comprising the step of transforming mammalian and other vertebrate ovarian host cells with GDF-9B and GDF-9 expression cassettes to allow over-expression of GDF-9B homodimers and GDF-9B/GDF-9 heterodimers.

According to a further aspect the invention provides a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15, or a functional fragment of variant of said sequence.

According to a further aspect the invention provides a polypeptide comprising an amino acid sequence said from the group consisting of SEQ ID NO: 12 and SEQ ID NO:16, or a functional fragment or variant of said sequence.

According to another aspect the invention provides a method of altering follicular growth comprising the step of introducing a ligand as claimed in any one of claims 9-15 to:

i) alter follicular growth in ovaries of a mammal or other vertebrate either *in vivo* or *in vitro; or*

ii) alter isolated ovarian cell growth/maturation *in vitro*

Preferably said mammal is selected from the group comprising sheep, cattle, goats, deer, pigs, humans, horses, camelids and possums, cats and dogs and any other commercially important species having a GDF-9B gene having a substantial identity to the GDF-9B sequences of the present invention. Said vertebrate is preferably selected from the group comprising chickens, ducks, geese, salmon and any other commercially important species having a substantial identity to the GDF-9B sequences of the present invention.

Brief Description of Drawings

In particular, preferred aspects of the invention will be described in relation to the accompanying drawings, in which:

Figure 1. Shows the nucleotide sequence of wildtype GDF-9B in sheep.

Figure 2. Shows a portion of the nucleotide sequence of Figure 1 and illustrates the Inverdale mutation.

Figure 3. Shows a portion of the nucleotide sequence of Figure 1 and illustrates the Hanna mutation.

Figure 4. Shows the deduced amino acid sequence of wildtype GDF-9B in sheep.

Figure 5. Shows a portion of the amino acid sequence of Figure 1 and illustrates the Inverdale mutation.

Figure 6. Shows a portion of the amino acid sequence of Figure 1 and illustrates the Hanna mutation.

Figure 7. Show the signal sequence polymorphism for GDF-9B with sheep.

Figure 8. Shows the additional upstream ATG codon present in sheep.

Figure 9. Shows photomicrograph illustrating the localisation of GDF-9B in an oocyte.

DETAILED DESCRIPTION OF THE INVENTION

As discussed above, the primary focus of the invention is the modulation of ovarian follicular growth via GDF-9B homodimers and GDF-9B/GDF-9 heterodimers activity *in vivo* and *in vitro*.

The term "isolated" means substantially separated or purified from contaminating sequences in the cell or organism in which the nucleic acid naturally occurs and includes nucleic acids purified by standard purification techniques as well as nucleic acids prepared by recombinant technology, including PCR technology, and those chemically synthesised.

Preferably, the nucleic acid molecule of SEQ ID NO: 1 is isolated from sheep genomic DNA, and that of SEQ ID NO: 3 and SEQ ID NO: 5 is isolated from DNA of sheep expressing the Inverdale or Hanna phenotype.

It has been noted that a polymorphism may occur in the signal peptide of GDF-9B in sheep (SEQ ID NO: 7, SEQ ID NO: 8). The predicted signal sequence appears to be around 25 amino acids long as predicted using the Signal P programme (Signal P V1.1 server at http://genome.cbs.dtu.dk/services/SignalP) (Neilsen et al., 1997) from ATG (Met) to ACA (Thr) in SEQ ID NO: 7 and SEQ ID NO: 8. A 3 base pair deletion is observed in some sheep where one of the two CTT sequences is not present. Therefore some sheep have a shorter signal sequence although most have the full length. From studies of Hanna, Hanna cross Inverdale, Inverdale and wild-type sheep either related or unrelated to Inverdale or Hanna, most were homozygous for two CTT's, although some were heterozygous for one CTT The prevalence of the shorter version of the signal peptide was found to be high in the Merino breed of sheep but low in Romney breeds indicating that the polymorphism may be related to breed. Most Romney sheep carried the longer signal sequence regardless of their carrier status for Inverdale or Hanna. Although this polymorphism needs to be acknowledged it does not effect or modify the claims made in this invention.

It has also been noted that an additional 'in-frame' ATG start codon is present in the sheep genomic DNA sequence 27 nucleotides upstream from the GDF-9B ATG start codon (SEQ ID NO: 9 , SEQ ID NO: 10). This sequence is present in all sheep sequenced, whether wildtype, Inverdale or Hanna carriers, and is clearly independent of the Inverdale and Hanna mutations. It is not known whether this upstream start codon is used during protein translation in sheep (SEQ ID NO: 9, SEQ ID NO: 10). If so it would result in a signal sequence of an additional 9 amino acids. Such a difference between sheep and other mammalian GDF-9B proteins would be unlikely to affect the function of the mature protein as this portion of the molecule is cleaved off in the active mature GDF-9B, but we mention the possibility of an alternative protein translation start site which may be present in sheep. The signal peptide prediction programme (Neilsen et al., 1997) indicates that this additional 9 amino acid sequence may function as a signal peptide and that the likely end site for the signal peptide would still be the Thr (T) amino acid indicated in SEQ ID NO: 7 and SEQ ID NO; 8.

In a further aspect, the present invention provides an isolated polypeptide selected from the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6 or a functional variant thereof which functions to manipulate ovarian follicular growth in a female mammal.

The polypeptide may be produced by expression of a suitable vector comprising the nucleic acid molecule of the invention or a functional variant thereof, in a suitable host cell as would be understood by a person skilled in the art.

The term "variant" as used herein refers to nucleotide and polypeptide sequences wherein the nucleotide or amino acid sequence exhibits substantially 50% or greater homology with the nucleotide or amino acid sequence of SEQ ID NOS: 1-6 respectively, preferably 75% homology and most preferably 90-95% homology to the sequences of the present invention: provided said variant has a biological activity as defined herein. The variant may be arrived at by modification of the native nucleotide or amino acid sequence by such modifications as insertion, substitution or deletion of one or more nucleotides or amino acids or it may be a naturally occurring variant. The term "variant" also includes homologous sequences which hybridise to the sequences of the invention under standard or preferably stringent hybridisation conditions familiar to those skilled in the art. Examples of the in situ hybridisation procedure typically used are described in (Tisdall et al.,1999); (Juengel et al.,2000). Where such a variant is desired, the nucleotide sequence of the native DNA is altered appropriately. This alteration can be made through elective synthesis of the DNA or by

modification of the native DNA by, for example, site-specific or cassette mutagenesis. Preferably, where portions of cDNA or genomic DNA require sequence modifications, site-specific primer directed mutagenesis is employed, using techniques standard in the art.

A "fragment" of a nucleic acid is a portion of the nucleic acid that is less than full length, and comprises at least a minimum sequence capable of hybridising specifically with a nucleic acid molecule according to the invention, or a sequence complementary thereto, under stringent conditions as defined below. A "fragment" of a polypeptide is a portion of the polypeptide which is less than full length, but which still retains the biological activity as defined herein.

The term "biologically active" refers to a polypeptide of the invention which is able to elicit a measurable physiological effect in the ovary or ovarian cell of a mammal or other vertebrate. The physiological effects may be measured by assays such as the incorporation of tritiated thymidine into granulosa cells. An example of such an assay is one in which follicles (1-2.5 mm diameter) are dissected free from ovarian stroma and granulosa cells isolated from theca and oocyte cumulus complexes. The cells are washed and resuspended in fresh media at a final concentration for bioassay of 100,000 viable cells per well and incubated with or without polypeptide for 48 hours. At this time incorporation of tritiated thymidine is measured.

The term "protein (or polypeptide)" refers to a protein encoded by the nucleic acid molecule of the invention, including fragments, mutations and homologs having the same biological activity i.e. ovulation manipulation activity. The polypeptide of the invention can be isolated from a natural source, produced by the expression of a recombinant nucleic acid molecule, or can be chemically synthesised.

The term "ligand" refers to any molecule which can bind to another molecule such as a polypeptides or peptide, and should be taken to include, but not be limited to, antibodies, cell surface receptors or phage display molecules.

In addition, nucleotides and peptides having substantial identity to the nucleotide and amino acid sequences of the invention can also be employed in preferred embodiments. Here "substantial identity" means that two sequences, when optimally aligned such as by the programs GAP or BESTFIT (peptides) using default gap weights, or as measured by computer algorithms BLASTX or

BLASTP, share at least 50%, preferably 75%, and most preferably 90-95% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to effect the properties of a protein. Non-limiting examples include glutamine for asparagine or glutamic acid for aspartic acid.

In a further aspect, the present invention provides in replicable transfer vectors suitable for use in preparing a polypeptide or peptide of the invention. These vectors may be constructed according to techniques well known in the art, or may be selected from cloning vectors available in the art.

The cloning vector may be selected according to the host or host cell to be used. Useful vectors will generally have the following characteristics:

(a) the ability to self-replicate;
(b) the possession of a single target for any particular restriction endonuclease; and
(c) desirably, carry genes for a readily selectable marker such as antibiotic resistance.

Two major types of vector possessing these characteristics are plasmids and bacterial viruses (bacteriophages or phages). Presently preferred vectors are bacterial, insect or mammalian vectors and may include the following: the pUC, pBlueScript, pGEM, PGEX, pBK-CMV, lambda ZAP, lambda GEM, pEFIRES-P, pUB6/V5/His, pBC1, pADTrack-CMV, pAdenovator, pAdEasy-1, pSFV-PD, pCA3, pBABE, pPIC9, pA0815, pET and pSP series. However, this list should not be seen as limiting the scope of the present invention.

Examples of preferred expression systems are as follows:

1. For an *in vitro* cell expression system, the 293T cell system with a pEFIRES-P vector (Hobbs S *et al.*,1998) which confers puromycin resistance may be used. For coexpression of two genes, the aforementioned vector may be modified to change the antibiotic resistance gene to bleomycin resistance. Alternatively, the co-expression of two genes and the selection gene can be achieved by constructing a tricistronic expression vector. A corresponding stably transfected insect cell system can also be used, e.g. the S2 cell system using "DES" vector expression system; www.invitrogen.com.

2. With respect to expressing GDF's in all tissues of transgenic animals, one approach is to use the pUB6/V5-His A vector (www.invitrogen.com) to make the constructs. For tissue-specific expression the rat PEPCK 0.6 kb promoter for liver and kidney expression can be included in the construct by replacing the Ubi-C promoter in the pUB6/V5-His A vector with the PEPCK promoter. For GDF expression in mammary tissue another promoter system would be preferred. For this tissue one approach would be to use the bovine β-lactoglobulin gene promoter and/or the bovine α S1 casein promoter (e.g. pBC1 vector, www.invitrogen.com) to drive the expression of the GDFs into milk. For global over-expression in transgenic animals, the CMV enhanced β-actin promoter (Okabe M, et al.; FEBS Letters 407: 313-319, 1997) or a modified EF1 α-promoter can be used also (Taboit-Dameron F, et al., Transgenic Research 8: 223-235, 1998).

Adenoviruses, retroviruses and alphaviruses are other suitable mammalian expression systems. A typical approach to those skilled in the art is that described by (TC He *et al.*, 1998),. With respect to GDF expression the pAd Track-CMV vector or pAdenovator vectors (www.qbiogene.com) can be used to make the construct which is then co-transformed with pAd Easy-1 adenoviral plasmid into *E. coli* to generate a recombinant adenoviral genome which contains a CMV-promoter driven GDF expression cassette. This recombinant adenoviral genome is then transfected into 293T cells to make the virus stock. Alternative methods for generating adenoviruses can also be used for the same purpose (e.g. PCA3 plasmid based gene transfer (www.microbix.com); or COS-TPC method (Miyake S *et al.*,1996).

3. Non-cytopathogenic Semliki Forest viruses expressing GDF's can be generated using, for example, pSFV-PD vectors as described by Lundstrom et al., Histochem Cell Biol 115: 83-91, 2001. Furthermore, retroviral expression systems based on, for example, pBABE vectors, can be used for expressing GDF's in mammalian cells (Morgenstern, JP and Land, H, 1990; Nucleic Acids Res 18: 3587-3596).

4. Yeast cells (e.g. *Pichia pastoris*, *Saccharomyces cerevisiae*) are another well established expression system to those skilled in the art (C Hadfield, *et al.*, 1993);(MA Romanos *et al.*, 1992),. For example, the pPIC9 vector (www.invitrogen.com) can be used in *Pichia pastoris* for the expression of GDF's. For coexpression of two genes, the vector pA0815 (www.invitrogen.com) is a preferred candidate.

5. *Echerichia coli* (*E. coli*) is a standard laboratory expression system in widespread use. For example, the pET expression system (www.novagen.com) can be used to express recombinant mammalian GDF-9 and GDF-9B (steve.lawrence@agresearch.co.nz).

The DNA molecules of the invention may be expressed by placing them in operable linkage with suitable control sequences in a replicable expression vector. Control sequences may include origins of replication, a promoter, enhancer and transcriptional terminator sequences amongst others. The selection of the control sequence to be included in the expression vector is dependent on the type of host or host cell intended to be used for expressing the DNA as would be understood by a person skilled in the art.

The expression vectors useful in the present invention may contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the glycolytic promoters of yeast acid phosphatase, e.g. Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, and cytomegalovirus e.g. the early and late promoters of SV40, and other sequences known to control the expression of genes of prokaryotic and eukaryotic cells and their viruses or combinations thereof.

In the construction of a vector it is also an advantage to be able to identify the bacterial clone carrying the vector incorporating the foreign DNA. Such assays include measurable colour changes, antibiotic resistance and the like. In one preferred vector, the β-galactosidase gene is used, which gene is detectable by clones exhibiting a blue phenotype on X-gal plates. This facilitates selection. Once selected, the vectors may be isolated from the culture using standard procedures.

Depending on the host used, transformation and transfection is performed according to standard techniques appropriate to such cells. For prokaryotes or other cells that contain substantial cell walls, the calcium treatment process (Cohen, S N Proceedings, National Academy of Science, USA 69 2110 (1972)) may be employed. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graeme and Van Der Eb, Virology 52:546 (1978) or liposomal reagents are preferred.

Upon transformation of the selected host with an appropriate vector the polypeptide encoded can be produced, often in the form of a fusion protein, by culturing the host cells. The polypeptide of the invention may be detected by rapid assays as indicated above. The polypeptide is then recovered and purified as necessary. Recovery and purification can be achieved using any procedures known in the art, for example by absorption onto and elution from an anion exchange resin. This method of producing a polypeptide of the invention constitutes a further aspect of the present invention.

Host cells (including whole animal hosts), transformed, transfected or infected with the vectors of the invention also form a further aspect of the present invention.

In addition, a further aspect of the present invention provides for an antibody, antibody fragment, single cell surface receptor or complex cell surface receptor or phage display molecules binding to a polypeptide or peptide of the invention. The polypeptide or peptide may be present as a monomer, dimer, heterodimer, multimer or a variant thereof.

More specifically, the invention provides methods for producing antibodies against the wild-type (SEQ ID NO: 2) or mutated (SEQ ID NO: 4 and SEQ ID NO: 6) GDF-9B polypeptide sequences as monomers or homodimers or as heterodimers in combination with GDF-9. The antibodies can be used for the characterisation of the wild-type endogenous proteins, or peptide fragments and expressed recombinant proteins, or peptide fragments and for passive immunisation of recipient mammals for the modulation of ovarian follicular growth *in vivo*.

It will be appreciated by the reader that a further aspect of the invention contemplates the use of the polypeptides of the invention in the preparation of antisera for the detection of other GDF-9B –like peptides.

Polyclonal antibodies may be produced according to the method used by (Koelle *et al.*, 1991) incorporated herein by reference. Useful antibody production protocols are outlined in US Patent 5,514,578. Monoclonal antibodies may be produced by methods known in the art. These methods include the immunological method described by (Kohler and Milstein, 1975) as well as the recombinant DNA method described by (Huse *et al.*, 1989).

In a further embodiment, the invention provides a method of manipulating ovarian follicular growth in cells in culture and/or transgenic animals. By overexpression of GDF-9B homodimers and GDF-9B/GDF-9 heterodimers, the timing and level of expression of specific ovarian follicular protein genes may be altered in cultured cells or transgenic animals, for example GDF-9B homodimers or GDF-9B/GDF-9 heterodimers encoding sequences may be inserted into a gene cassette under the control of a specific promoter or a promoter that expresses in all cell types (see examples below) (constitutive expression). This cassette also comprises 3' flanking DNA that could stabilise the mRNA and may optionally comprise downstream regulatory sequences. This DNA cassette could be introduced into the genome of mammals by micro injection of the DNA into the pronuclei of eggs (as described in L'Huillier *et al.*, 1996) which are subsequently transferred back to recipient animals and allowed to develop to term. This technique for the production of transgenic animals is described by (Hogan *et al.*,1996). Transgenic animals may be produced by transfection of cells in culture derived from an embryo, or foetal or adult tissues; followed by nuclear transfer and embryo transfer to recipient animals. Alternatively the gene cassette may be bound to mammalian sperm and delivered to the egg via *in vitro* or *in vivo* fertilisation to produce a non-human transgenic animal. Manipulation of the developmental regulation or the level of expression of GDF-9B homodimers or GDF-9B/GDF-9 heterodimers may be used to alter the level of ovarian follicular protein synthesis or production.

The invention also includes adenovirus-based gene therapy techniques for expressing GDF-9B and GDF-9/GDF-9B in cell cultures, organ cultures and whole experimental animals for manipulating ovarian follicular protein synthesis or production.

Non-limiting examples illustrating the invention will now be provided. It will be appreciated that the above description is provided by way of example only and variations in both the materials and techniques used which are known to those persons skilled in the art are contemplated.

EXAMPLES

EXAMPLE 1

Isolation of wildtype ovine GDF-9B DNA and identification of mutated ovine GDF-9B DNA sequences

Different combinations of oligonucleotide primers derived from human/mouse/rat GDF-9B sequences previously cloned by us were used in PCR on genomic sheep DNA for obtaining fragments of the ovine GDF-9B gene for sequencing. Functional primer pairs were used for obtaining wild-type sheep genomic clones from arrayed libraries and for obtaining cDNA sequence from wild-type ovine ovarian cDNA. The sequence from the full coding regions of Inverdale and Hanna was obtained by sequencing relevant PCR fragments obtained from the respective genomic DNA's. The wildtype GDF-9B sequence is disclosed here as SEQ ID NO: 1, Inverdale as SEQ ID NO: 3, and Hanna as SEQ ID NO: 5.

EXAMPLE 2

Production of specific antibodies and demonstration of natural homodimeric GDF-9B and heterodimeric GDF-9B/GDF-9 proteins in mammalian ovaries.

Antigens for immunising mammals or birds are generated using nucleotide sequences disclosed in the invention as SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5 or variants thereof in operative association with an expression control sequence enabling expression of the protein in E. coli. Yet as another approach, anti-peptide antibodies directed against specific peptide sequences from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or variants thereof are generated. Immunoreactivity can be assessed by standard methods (e.g. ELISA) and/or to obtain specific IgGs recognising GDF-9B homodimers and GDF-9B/GDF-9 protein heterodimers from natural sources and cell/tissues overexpressing the respective recombinant proteins.

Previous evidence by the inventors' group (e.g. Aaltonen *et al.* (1999) and Jaatinen *et al.*, 1999) show the presence of GDF-9 mRNA and protein in oocytes of preantral follicles in rodents and primates. Here we show evidence by immunocytochemistry using a mouse antibody for the presence of GDF-9B protein in sheep ovaries (Fig. 9). The immunohistochemical methodology was similar to that described by Tisdall *et al.* (1999) Stem cell factor and c-kit gene expression and protein localisation in the sheep ovary during fetal development, J Reprod Fert 116: 277-291. The only exception was that a tyramide signal amplification step was also included in the present study (TSA Biotin System, NEN Life Science Products). The mouse antibody M10 was generated after an *E. coli* derived mature ovine GDF-9B peptide (0.2 mg) was injected in Freunds complete adjuvant (FCA), intraperitoneally (i.p.) and at 2 weekly intervals boosted with 0.1 mg antigen i.p.

and subsequently with 0.05 mg antigen in a Span/Tween/oil mixture and the animal sacrificed 1 week after the final booster and serum collected.

Collectively these and other data show that both the mRNA and protein for GDF-9 and GDF-9B are present in oocytes of mammals (Aaltonen *et al.*, 1999; Galloway *et al.*, 2000).

Evidence that antipeptide antibodies directed against specific peptide sequences affect mammalian ovarian activity is shown in Table 1.

Table 1: Ovulation rate in sheep following administration of plasma containing antibodies to keyhole limpet haemocyanin (KLH) conjugated to GDF-9B peptide

Treatment	Mean Ovulation Rate (range)
Anti-KLH (n=5 animals)	1.6 (1-2)
Anti-GDF-9B peptide (5)	0.2* (0-1)

*$p<0.05$ (students t-test)

In this study 10 female sheep had their oestrous (i.e. ovarian) cycles synchronised using prostaglandin $F_{2\alpha}$ (Estrumate, 125 μg i.m.). All animals were observed to show synchronised oestrus following markings with vasectomised rams. On day 5 of the subsequent luteal phase, 5 ewes were administered with a pooled plasma recovered from another flock of ewes that had been immunised on 7 consecutive monthly occasions with a 15 mer GDF-9B peptide conjugated to keyhole limpet haemocyanin (KLH). The pooled sera contained high titre antibodies to GDF-9B as assessed by ELISA assay using a full-length *E. coli* expressed GDF-9B as antigen. The other 5 ewes were administered with a pooled plasma to KLH recovered from a separate flock of ewes that had been immunised also on 7 consecutive monthly occasions. The pooled plasma from these animals did not contain detectable GDF-9B antibody.

Antibody levels were measured by an ELISA procedure after the sheep plasmas were diluted 1:50000. The ELISA method involved coating a 96-well plate with 100 ng/well of an *E. coli*

expressed full-length GDF-9B and incubation with 100 µl of diluted sheep plasma and 100 µl of assay buffer, after appropriate blocking treatment and successive washes. After incubation with the sheep plasma and several washes, rabbit anti-sheep-HRP was added for 1 h at 37°C. The wells were then washed and developed with o-phenylenediamine plus hydrogen peroxide with development being stopped with sulphuric acid.

The ewes were each given 100 ml of sterile plasma intravenously and 4 days later administered with a second prostaglandin $F_{2\alpha}$ injection to synchronise oestrus. The ovulation rates were examined by laparoscopy 14 days after administration of plasma.

In our previous patent no. 500844 we demonstrated a significant perturbation in ovarian follicular development in mice when the animals were immunised with an *E. coli* derived mature ovine GDF-9B. In this study 10 female mice were immunised intraperitoneally (i.p.) with the *E. coli*-derived mature ovine GDF-9B protein (0.2 mg) in Freunds complete adjuvant (FCA) (0.2 ml), and another 10 female mice were immunised with bovine alpha lactalbumin (0.2 mg) in FCA (0.22 ml) i.p. to serve as controls. Subsequently, 3 booster injections of the appropriate antigens (0.1 mg at first booster and 0.05 mg at second and third booster) were given at 2 week intervals in a Span/Tween/oil mixture and the animals sacrificed 1 week after the final booster.

Here we provide evidence for these GDF-9B immunised mice that the exogenous biological activities of both GDF-9 and GDF-9B are likely to be affected since these animals contained cross-reacting antibodies to both growth factors (Table 2).

Table 2: Mean (± s.e.m.) antibody levels in mouse plasma following immunisation with bovine alpha lactalbumin or ovine GDF-9B. The values presented show the absorbance at 490 nm which represents the levels of antibody to GDF-9B or GDF-9

Treatment	GDF-9B	GDF-9
Bovine α-lactalbumin	<0.060	<0.060
GDF-9B	2.177 (±0.163)	0.590 (±0.058)

Thus we assert that procedures that lead to the modulation of endogenous GDF-9 and GDF-9B will alter ovarian function.

Further evidence in support of our claim that modulating homodimeric GDF-9B or GDF-9 and GDF-9B either as homodimeric mixtures or heterodimers is provided by our novel findings following the immunisation of sheep (3-5 per treatment group) against KLH (control), GDF-9B peptide conjugated to KLH or GDF-9 peptide conjugated to KLH. The animals were subjected to 7 consecutive monthly immunisations, the ovaries recovered after slaughter and thereafter the ovarian volumes and numbers of types 1, 1a and 2 follicles were assessed by standard morphometric procedures (Smith *et al.*, 1997). In addition the presence or absence of antral follicles were noted. These results are summarised in Table 3.

Table 3: Mean ovarian volumes and numbers of Types 1-2, 3-4 and antral follicles following immunisation of sheep with KLH, KLH conjugated to GDF-9B peptide or to GDF-9 peptide

Treatment	Ovarian volume (mean range)	Number of Type 1-2 follicles (mean range)	Number of Type 3-4 follicles (mean range)	Number of antral follicles (mean range)
KLH (3)	486 (353-651)	15180 (11623-17433)	63 (61-65)	28 (22-37)
GDF-9B Peptide-KLH (5)	296 (149-488)	8147 (3221-13786)	0	0
GDF-9 Peptide-KLH (2)	284 (248-303)	16278 (10152-25745)	0	4 (0-11)

For these studies the GDF-9B peptide sequence was SEVPGPSREHDGPESC (SEQ ID NO 17) and the GDF-9 peptide sequence was KKPLVPASVNLSEYFC (SEQ ID NO 18). The Romney ewes were injected with 0.4 mg/ewe of KLH or KLH-GDF-9B peptide or KLH-GDF-9 peptide in Freund's complete adjuvant. Subsequently at monthly intervals on 6 occasions, the animals were boosted with further antigen (0.2 mg/ewe on each occasion) in a Span/Tween/oil mixture (s.c.).

The results show that relative to the controls (KLH immunisations): (1) GDF-9-KLH and GDF-9B-KLH immunisations each can inhibit antral follicle development thereby demonstrating that both GDF-9 and GDF-9B are essential for normal follicular development in some mammals.

EXAMPLE 3

Expression of recombinant homodimeric GDF-9B and heterodimeric GDF-9B/GDF-9 protein in mammalian cells *in vitro* and *in vivo*.

Expression constructs generated in plasmid vectors containing nucleotide sequences disclosed in the invention as SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5 or variants thereof in operative association with an expression control sequence (CMV, EF1, and mammary specific promoter sequences) are transfected/transferred to mammalian cells and stable clones are selected using an antibiotic selection marker. For optimising the processing of the expressed recombinant polypeptide sequence the furin processing site is mutated and an auxiliary expression cassette driving the overexpression of the furin protease in the producer cells is introduced.

As an example of an effective mammalian expression system for producing oGDF-9B homodimeric protein we have generated human 293T cell lines transfected with the pEFIRES-P vector containing the rat GDF-9B proregion (Jaatinen et al., Mol Cell Endocrinol. 156:189-93, 1999) fused to an ovine GDF-9B mature region sequence (SEQ ID NO: 1 and NO:2). The furin processing site had been engineered to contain the effectively cleaved RRRR sequence. Cells selected to resist 120-150 ug/ml puromycin were cultured in HamF12/DMEM under serum free conditions for 4 days to produce ovine GDF-9B into the supernatant which was subsequently used in bioassay as described in Example 4.

To gain biochemical evidence for physical heterodimerisation of sheep GDF-9B with GDF-9 the following approach was used. A similar rat/sheep chimeric GDF-9B open reading frame as described above but containing a C-terminal 8 amino acid FLAG epitope was cloned into pSFV-PD Semliki Forest Virus vector and a high titre virus stock of SFV-PD-oGDF-9B-FLAG was generated in BHK cells as described in Lundstrom et al, Histochem Cell Biol 115: 83-91, 2001). A high titre SFV-PD-oGDF-9B-FLAG virus was seen to very effectively infect human 293T cells and cause high expression of oGDF-9B-FLAG in these cells. After infection, during a 4 day culture processed oGDF-9B-FLAG was secreted to medium and the protein was easily visualised as a 18 kd band in Western blot analysis using anti-FLAG M2 antibody. For co-expression of ovine GDF-9B-FLAG with ovine GDF-9 another 293T cell line was generated using a pEFIRES-P vector containing an open reading frame encoding a prepro GDF-9 polypeptide. Parental 293T cells and 293T cells stably

expressing oGDF-9 were infected with equal amounts of SFV-PD-oGDF-9B-FLAG viruses and supernatants were collected after 4 days in culture. One ml of supernatants from uninfected and SFV-PD-oGDF-9B-FLAG infected cells were subjected to immunoprecipitation with 1 μg/ml anti FLAG-M2 antibodies and complexes were recovered with protein G agarose. Eluates were evaluated in Western blots using anti-FLAG-M2 antibodies and anti GDF-9 antibodies. Although anti FLAG M2 antibodies do not react with ovine GDF-9, an immunoreactive 20 kd GDF-9 mature peptide was seen in supernatants of GDF-9 expressing cells that had been infected with SFV-PD-oGDF-9B-FLAG viruses and immunoprecipitated with anti FLAG M2 antibodies. These co-immunoprecipitation experiments indicate a direct physical interaction of recombinantly expressed ovine GDF-9B and GDF-9 and confirm the existence of sheep GDF-9/GDF-9B heterodimers.

Methods are described for over-expressing nucleotide sequences disclosed in the invention as SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5 or variants thereof in several extraovarian sites in transgenic animals for mimicking systemic administration of recombinant homodimeric GDF-9B and heterodimeric GDF-9B/GDF-9 proteins. The relevant GDF encoding sequences are either expressed separately or co-expressed in operative association with expression control sequences. Effects of GDF-9B homodimers and GDF-9B/GDF-9 heterodimers on ovarian follicular growth in these transgenic animals are assessed by morphometric measurements or hormonal assays. This approach provides a general method for altering ovulation rate in transgenic animals. Production of GDF's into milk by mammary gland directed overexpression provides an alternative method of producing large amounts of reagent for medical or pharmaceutical purposes. This approach is not injurious to the health of the transgenic animal as no contraindications have been observed.

A method is described for effectively transferring an expression cassette composed of nucleotide sequences disclosed in the invention as SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5 or variants thereof in operative association with an expression control sequence (CMV promoter) in a recombinant adenovirus into recipient cell or organ cultures or recipient animals for altering follicular growth *in vitro* or *in vivo*. For example, the methods allow systemic infection of recipient mammals with GDF-9B and GDF-9 expression cassette bearing adenoviruses concentrating into the livers of the host and allowing high levels of liver derived recombinant proteins to be released to the circulation. Effects on ovarian follicular growth may be assessed by one or more criteria such as laparoscopy, morphometric measurements or hormonal assays. A similar *in vivo* viral transfer of GDF-9B And GDF-9 sequences and transcriptional units can be achieved with alphaviruses or

retroviruses. These viral approaches offer ways of testing the bioactivity of different GDF-9B and GDF-9 gene constructs *in vivo* and also provide alternative ways of immunising animals against GDF-9B and GDF-9.

EXAMPLE 4

Measurement of the biological activity of homodimeric GDF-9B and heterodimeric GDF-9B/GDF-9 proteins in ovarian cell cultures.

For assessing the biological activity of proteins expressed from the nucleotide sequences disclosed in the invention as SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5 or variants thereof ovarian cell and organ culture models may be used.

An example of assessing the biological activity of homodimeric GDF-9B is shown in Table 4 below whereby a partially purified recombinant (r) ovine (o)GDF-9B extract is assessed by measurement of [^{3}H] thymidine incorporation by isolated ovine granulosa cells during a 48 h incubation at 37°C. The results show that the roGDF-9B caused a 1.9-fold increase in tritiated thymidine incorporation by granulosa cells indicating that the roGDF-9B was biologically active. To obtain the granulosa cells, ovaries were recovered from ewes, follicles (1-2.5 mm diameter) dissected free and the cells isolated and separated from theca and oocyte-cumulus complexes. The cells were washed and resuspended in fresh media at a final concentration for bioassay of $1x10^5$ viable cells per well.

Table 4: Mean ± s.e.m. increase in [^{3}H] thymidine incorporation in ovine (o) granulosa cells after exposure to roGDF-9B or control media (n=3 separate experiments)

Treatment	[^{3}H] thymidine incorporation (cpm)
Control	3256±283
Rec ovine (o)-GDF-9B	6291±503***

RoGDF-9B was produced by transfected 293T cells and partially purified using heparin-sepharose chromatography and the protein eluted with 0.5 M NaCl and dialysed overnight against tissue culture media. The control for this experiment was media exposed to non-transfected 293T cells and subjected to heparin-sepharose chromatography, NaCl elution and dialysis.

***$p<0.001$, ANOVA

DISCUSSION

The known human GDF-9B sequence and oligonucleotide primers derived thereof enabled the inventors to determine the sheep GDF-9B genomic and cDNA sequences and to assess the expression of GDF-9B transcripts in sheep ovaries (Galloway et al., 2000).

From literature it appears that GDF-9 is needed for granulosa cell mitogenesis and thecal cell differentiation. Indeed, recombinant rat GDF-9 is able to stimulate rat follicular growth in vitro (Hayashi et al., 1999) as well as the proliferation of rat granulosa cells in culture (Vitt et al., 2000). Recombinant GDF-9 also regulates steroidogenesis and gonadotrophin receptor expression in mouse and rat granulosa cells (Elvin et al., 1999; Vitt et al., 2000). Furthermore, GDF-9 stimulates inhibin B production in human granulosa cells in culture (Vuojolainen et al., in preparation). These recent studies clearly demonstrate that GDF-9 homodimers have potent effects on follicular growth and differentiation in several mammals but before the invention described herein nothing has been known on the possible biological effect of GDF-9B in the ovary.

In our earlier New Zealand patent specification no. 500844 the present inventors showed that the Inverdale gene maps to a sheep X-chromosome region containing genes syntenic to Xp11.2-11.4 in human (Galloway *et al.*, 2000) and determined whether GDF-9B gene is affected in these animals, showing that the Inverdale gene is actually an inactivated form of the sheep GDF-9B gene. In Inverdale animals the T nucleotide at position 92 nucleotides beyond the mature peptide processing site has become an A residue, converting the codon GTC to GAC causing the substitution of the amino acid valine (V) to aspartic acid (D). In all TGF-β family members this very amino acid is either valine, isoleucine or leucine which all represent hydrophobic residues in contrast to the negatively charged aspartic acid. This amino acid substitution causes a change in the surface charge of the very area of the molecule which is involved in the dimerisation process as suggested from the crystal structures of TGF-β 2, BMP-2 and BMP-7 (Schlunegger and Grutter, 1993; Griffith et al., 1996; Scheufler et al., 1999). The invention of New Zealand 500844 also provides evidence for a second GDF-9B gene mutation identified in another flock of sheep, Hanna, having exactly the same heterozygous and homozygous gene carrier phenotypes as Inverdale ewes. Hanna animals present with a C to T nucleotide point mutation at position 67 nucleotides beyond the mature peptide processing site introducing a premature stop codon at the place of a glutamine (Q) residue. This change will cause a major truncation of the mature peptide region causing inactivation of the protein

product. Crosses between the Inverdale and Hanna families lead to 50% infertile females proving that both mutations clearly inactivate the GDF-9B gene product.

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WHAT WE CLAIM IS:

1. An isolated wildtype GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
   a) SEQ ID No:1;
   b) a sequence able to hybridise under stringent conditions to the molecule in (a);
   c) a sequence which is a functional variant or fragment of the molecule in (a);
   d) a sequence complementary to the molecule defined in (a), (b) or (c); and
   e) an anti-sense sequence corresponding to any of the molecules in (a) – (d).

2. An isolated full length mutated GDF-9B nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
   a) SEQ ID NO: 3 or SEQ ID NO: 5 ;
   b) a sequence able to hybridise under stringent conditions to the molecule(s) in (a);
   c) a sequence which is a functional variant or fragment of the molecule(s) in (a);
   d) a sequence complementary to the molecule(s) defined in (a), (b) or (c); and
   e) an anti-sense sequence corresponding to any of the molecule(s) in (a) – (d).

3. An isolated full-length GDF-9B polypeptide comprising an amino acid sequence selected from the group consisting of:
   a) SEQ ID NO:2 , SEQ ID NO:4 ; or SEQ ID NO:6; and
   b) A functional variant or fragment of the sequence(s) in (a).

4. A homodimeric mature GDF-9B polypeptide having subunits comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2 or a functional fragment or variant of said sequence.

5. A heterodimeric polypeptide having subunits selected from the group consisting of:
   a) A mature GDF-9B polypeptide comprising an amino acid sequence derived from SEQ ID NO:2 or a functional fragment or variant of said sequence; and
   b) A mature GDF-9 polypeptide or a functional variant or fragment thereof.

6. A vector comprising the nucleic acid molecule of claim 1 or claim 2.

7. A construct comprising the nucleic acid moleculeof claim 1 or claim 2.

8. A host cell which has been transformed by a vector or construct as claimed in claim 6 or claim 7.

9. A ligand which binds to a polypeptide derived from the full-length prepro polypeptide as claimed in claim 3.

10. A ligand which binds to a homodimeric polypeptide as claimed in claim 4.

11. A ligand which binds to a heterodimeric polypeptide as claimed in claim 5.

12. A ligand as claimed in claim 10 or 11, wherein the ligand is an antibody or an antibody fragment comprising the antigen-binding domain.

13. A ligand as claimed in claim 12 wherein the ligand is a monoclonal antibody.

14. A ligand as claimed in claim 10 or 11 wherein the ligand is a phage display molecule.

15. A ligand as claimed in claim 10 or 11 where the ligand is in the form cell surface receptor(s).

16. A method of expressing biologically active processed homodimeric GDF-9B polypeptide comprising the steps of:

a) generating an expression construct comprising a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 or a functional fragment or variant of said sequence of the group;
b) transfecting suitable cells with said construct;
c) selecting stable clones; and
d) isolating and purifying the expressed polypeptide.

17. A method of expressing biologically-active processed heterodimeric GDF-9B and GDF-9 polypeptides comprising the steps of:

a) generating an expression construct containing a nucleic acid molecule comprising:
   (i) a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1 or a functional fragment or variant of said sequence; and
   (ii) a nucleic acid molecule encoding GDF-9 or a functional fragment or variant thereof;
b) transfecting suitable cells with said construct;
c) selecting stable clones; and
d) isolating and purifying the expressed polypeptide.

18. A method of adenoviral, retroviral and alphaviral transfer of GDF-9B expression cassettes or GDF-9 expression cassettes to host cells or organisms to thereby effect *in vivo* expression of GDF-9B homodimers or GDF-9B/GDF-9 heterodimers, comprising the step of transferring into a recipient cell, organ culture or recipient animal, a recombinant adenovirus including an expression cassette comprising a nucleic acid molecule having a nucleotide sequence selected from the group consisting of SEQ ID NO:1 or a functional fragment or variant of said sequence, said nucleic acid molecule being in operative association with an expression control sequence.

19. A transgenic animal which has been transformed by a vector or construct as claimed in claim 6 or 7.

20. A method of altering ovarian follicular growth, *in vivo*, in a female mammal or other female vertebrate, said method comprising the step of transforming mammalian and other vertebrate ovarian host cells with GDF-9B and GDF-9 expression cassettes to allow over-expression of GDF-9B homodimers and GDF-9B/GDF-9 heterodimers.

21. A method of altering ovarian follicular growth, *in vitro*, in a female mammal or other female vertebrate, said method comprising the step of transforming mammalian and other vertebrate ovarian host cells with GDF-9B and GDF-9 expression cassettes to allow over-expression of GDF-9B homodimers and GDF-9B/GDF-9 heterodimers.

22. A method as claimed in claim 19 or 20 wherein said mammal is selected from the group consisting of sheep, cattle, goats, deer, humans, pigs, horses, camelids, and possums, cats and dogs and any other commercially important species.

23. A method as claimed in claims 19 or 20 wherein said vertebrate is selected from the group consisting of chickens, ducks, geese, salmon and any other commercially important species.

24. A composition comprising an effective amount of an agent selected from the group consisting of:

a) a homodimeric polypeptide having subunits comprising a GDF-9B polypeptide or a functional fragment or variant thereof with or without homodimeric polypeptide having subunits comprising GDF-9 polypeptide or a functional fragment or variant thereof;

b) a heterodimeric polypeptide having subunits comprising a GDF-9B polypeptide and a GDF-9 polypeptide, or functional fragments or variants of said GDF-9B or GDF-9 polypeptides;

together with a pharmaceutically or veterinarily acceptable carrier (including adjuvants) or diluent; and optionally including supplementary gonadotrophins.

25. The use of an agent selected from the group consisting of:

a) a homodimeric polypeptide having subunits comprising GDF-9B or a functional fragment or variant thereof with or without homodimeric polypeptide having subunits comprising GDF9 polypeptide or a functional fragment or variant thereof;

b) a heterodimeric polypeptide having subunits comprising GDF-9B and GDF-9 polypeptides, or functional fragments or variants of said GDF-9B or GDF-9 polypeptides;

together with or without supplementary gonadotrophins and/or other ovarian growth factors including IGF-1, kit ligand (stem cell factor), epidermal growth factor or TGFβ agonists/antagonists to:

i) alter follicular growth in ovaries of a mammal or other vertebrate either *in vivo* or *in vitro; or*

ii) alter isolated ovarian cell growth/maturation *in vitro.*

26. A method for assessing the activity of GDF-9B homodimers and/or GDF-9B/GDF-9 heterodimers, comprising the steps of:
    a) adding an effective amount of a GDF-9B homodimeric polypeptide; and/or a GDF-9B/GDF-9 heterodimeric polypeptide to an ovarian cell or organ culture with or without other ovarian growth factors (including TGFβ agonists/antagonists); and
    b) conducting a bioassay on said cell or organ culture to assess the biological activity of said homodimeric and heterodimeric polypeptides.

27. A method of altering follicular growth comprising the step of introducing a ligand as claimed in any one of claims 9-15 to:
    ii) alter follicular growth in ovaries of a mammal or other vertebrate either *in vivo* or *in vitro; or*
    iii) alter isolated ovarian cell growth/maturation *in vitro*

28. An isolated functional variant polypeptide comprising an amino acid sequence selected from this group consisting of SEQ ID NO: 17.

29. An isolated functional variant polypeptide comprising an amino acid sequence selected from this group consisting of SEQ ID NO: 18.

30. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15, or a functional fragment or variant of said sequences.

31. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 14 and SEQ ID NO: 16, or a functional fragment or variant of said sequences.

32. An isolated nucleic acid molecule substantially as described herein with reference to any example and/or drawing thereof.

33. An isolated polypeptide substantially as described herein with reference to any example and/or drawing thereof.

34. A vector or gene construct incorporating an isolated nucleic acid molecule of the present invention substantially as described herein with reference to any example and./or drawing thereof.

35. A ligand which binds to a polypeptide of the present invention substantially as described herein with reference to any example and/or drawing.

36. A homodimeric polypeptide substantially as described herein with reference to any example and /or drawing thereof.

37. A heterodimeric polypeptide substantially as described herein with reference to any example and /or drawing thereof.

38. A method of expressing a biologically active processed homodimeric polypeptide substantially as described herein with reference to any example thereof.

39. A method of expressing a biologically active processed heterodimeric polypeptide substantially as described herein with reference to any example thereof.

40. A method of adenoviral transfer of polypeptides to thereby effect *in vivo* expression of homodimeric or heterodimeric polypeptides of the invention substantially as described herein with reference to any example thereof.

41. A method of altering ovarian follicular growth in a female mammal or other vertebrate substantially as described herein in reference to any example thereof.

42. A composition substantially as described herein with reference to any example and/or drawing thereof.

43. The use of a homodimeric or heterodimeric polypeptide to alter follicular growth substantially as described herein with reference to any example thereof.

44. A method for assessing the biological activity of homodimeric and heterodimeric polypeptides substantially as described herein with reference to any example thereof.

Figure 1 Ovine GDF-9B (BMP-15) wild type nucleotide sequence. The initiating codon (ATG) and stop codon (TGA) are indicated in boldface lettering. Coding sequence is indicated in uppercase lettering, noncoding exon sequence is indicated in lowercase lettering and lowercase italicised lettering indicates intron sequence. The numbers refer to coding nucleotides beginning with the first nucleotide of the ATG initiating codon.

```
-234                    ctgc tgtttctgttt gtttgatgc aaagaggaca
-201  atttagaaga cctctttttg gttcaggaga tcctaccaga ggaagaaaca
-151  taggacctgc ctgccagcct ttcatttttc cttgccctat cctttgtggt
-101  agtggagcct ggatgctgtt acccatgtaa aaggaaaggt ttaaagcgtt
- 51  atcctttggg cttttatcag aacatgttgc tgaacaccaa gcttttcaag

  1  ATGGTCCTCC TGAGCATCCT TAGAATCCTT CTTTGGGGAC TGGTGCTTTT

 51  TATGGAACAT AGGGTCCAAA TGACACAGGT AGGGCAGCCC TCTATTGCCC

101  ACCTGCCTGA GGCCCCTACC TTGCCCCTGA TTCAGGAGCT GCTAGAAGAA

151  GCCCCTGGCA AGCAGCAGAG GAAGCCGCGG GTCTTAGGGC ATCCCTTACG

201  GTATATGCTG GAGCTGTACC AGCGTTCAGC TGACGCAAGT GGACACCCTA

251  GGGAAAACCG CACCATTGGG GCCACCATGG TGAGGCTGGT GAGGCCGCTG

301  GCTAGTGTAG CAAGGCCTCT CAGAGgtgag ttatcatact atattgttct

     ggtgggaggg ggggagaaaa tggggaagaa aagtgtagaa aaaagtggat
     ctgtcagttt tctgtcaggc ttcacattgc ctacagggta ggtggttttc
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.....intron approx 5.4.kb .....

.....

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taggatataa gaagctaacg ctttgctctt gttccctctt actaatgcag

326 GCTCCTGGCA CATACAGACC CTGGACTTTC CTCTGAGACC AAACCGGGTA

376 GCATACCAAC TAGTCAGAGC CACTGTGGTT TACCGCCATC AGCTTCACCT

426 AACTCATTCC CACCTCTCCT GCCATGTGGA GCCCTGGGTC CAGAAAAGCC

476 CAACCAATCA CTTTCCTTCT TCAGGAAGAG GCTCCTCAAA GCCTTCCCTG

526 TTGCCCAAAA CTTGGACAGA GATGGATATC ATGGAACATG TTGGGCAAAA

576 GCTCTGGAAT CACAAGGGGC GCAGGGTTCT ACGACTCCGC TTCGTGTGTC

626 AGCAGCCAAG AGGTAGTGAG GTTCTTGAGT TCTGGTGGCA TGGCACTTCA

676 TCATTGGACA CTGTCTTCTT GTTACTGTAT TTCAATGACA CTCAGAGTGT

726 TCAGAAGACC AAACCTCTCC CTAAAGGCCT GAAAGAGTTT ACAGAAAAAG

776 ACCCTTCTCT TCTCTTGAGG AGGGCTCGTC AAGCAGGCAG TATTGCATCG

826 GAAGTTCCTG GCCCCTCCAG GGAGCATGAT GGGCCTGAAA GTAACCAGTG

876 TTCCCTCCAC CCTTTTCAAG TCAGCTTCCA GCAGCTGGGC TGGGATCACT

926 GGATCATTGC TCCCCATCTC TATACCCCAA ACTACTGTAA GGGAGTATGT

976 CCTCGGGTAC TACACTATGG TCTCAATTCT CCCAATCATG CCATCATCCA

1026 GAACCTTGTC AGTGAGCTGG TGGATCAGAA TGTCCCTCAG CCTTCCTGTG

1076 TCCCTTATAA GTATGTTCCC ATTAGCATCC TTCTGATTGA GGCAAATGGG

1126 AGTATCTTGT ACAAGGAGTA TGAGGGTATG ATTGCCCAGT CCTGCACATG

1176 CAGGTGAcgg caaaggtgca

Figure 2 A portion of the sequence encoding GDF-9B (BMP15) in sheep carrying the Inverdale mutation. Numbering of nucleotides is the same as found in the wildtype sequence (Figure 1). The mutation, which causes a change in the primary amino acid sequence, is indicated with boldface type. This was the only difference consistently observed between wild-type sheep and those containing the Inverdale mutation in the GDF-9B gene, but see Figure 7, Figure: 8

776 ACCCTTCTCT TCTCTTGAGG AGGGCTCGTC AAGCAGGCAG TATTGCATCG

826 GAAGTTCCTG GCCCCTCCAG GGAGCATGAT GGGCCTGAAA GTAACCAGTG

876 TTCCCTCCAC CCTTTTCAAG ACAGCTTCCA GCAGCTGGGC TGGGATCACT

926 GGATCATTGC TCCCCATCTC TATACCCCAA ACTACTGTAA GGGAGTATGT

976 CCTCGGGTAC TACACTATGG TCTCAATTCT CCCAATCATG CCATCATCCA

Figure 3 A portion of the sequence encoding GDF-9B (BMP15) in sheep carrying the Hanna mutation. Numbering of nucleotides is the same as found in the wildtype sequence (Figure 1). The mutation, which causes a premature stop codon, is indicated in bold typeface. This was the only difference consistently observed between wild-type sheep and those containing the Hanna mutation in the GDF-9B gene, but see Figure 7, Figure 8.

726 TCAGAAGACC AAACCTCTCC CTAAAGGCCT GAAAGAGTTT ACAGAAAAAG

776 ACCCTTCTCT TCTCTTGAGG AGGGCTCGTC AAGCAGGCAG TATTGCATCG

826 GAAGTTCCTG GCCCCTCCAG GGAGCATGAT GGGCCTGAAA GTAACTAGTG

876 TTCCCTCCAC CCTTTTCAAG TCAGCTTCCA GCAGCTGGGC TGGGATCACT

926 GGATCATTGC TCCCCATCTC TATACCCCAA ACTACTGTAA GGGAGTATGT

976 CCTCGGGTAC TACACTATGG TCTCAATTCT CCCAATCATG CCATCATCCA

Figure 4 The deduced amino acid sequence of wildtype ovine GDF-9B (BMP-15) as determined from the wildtype nucleotide sequence of ovine GDF-9B (BMP-15).

1 MVLLSILRIL LWGLVLFMEH RVQMTQVGQP SIAHLPEAPT LPLIQELLEE

51 APGKQQRKPR VLGHPLRYML ELYQRSADAS GHPRENRTIG ATMVRLVRPL

101 ASVARPLRGS WHIQTLDFPL RPNRVAYQLV RATVVYRHQL HLTHSHLSCH

151 VEPWVQKSPT NHFPSSGRGS SKPSLLPKTW TEMDIMEHVG QKLWNHKGRR

201 VLRLRFVCQQ PRGSEVLEFW WHGTSSLDTV FLLLYFNDTQ SVQKTKPLPK

251 GLKEFTEKDP SLLLRRARQA GSIASEVPGP SREHDGPESN QCSLHPFQVS

301 FQQLGWDHWI IAPHLYTPNY CKGVCPRVLH YGLNSPNHAI IQNLVSELVD

351 QNVPQPSCVP YKYVPISILL IEANGSILYK EYEGMIAQSC TCR

Figure 5 The deduced amino acid sequence of ovine GDF-9B (BMP-15) in sheep carrying the Inverdale mutation as determined from the nucleotide sequence of ovine GDF-9B (BMP-15) in sheep carrying the Inverdale mutation. The single amino acid change is underlined in bold.

1 MVLLSILRIL LWGLVLFMEH RVQMTQVGQP SIAHLPEAPT LPLIQELLEE

51 APGKQQRKPR VLGHPLRYML ELYQRSADAS GHPRENRTIG ATMVRLVRPL

101 ASVARPLRGS WHIQTLDFPL RPNRVAYQLV RATVVYRHQL HLTHSHLSCH

151 VEPWVQKSPT NHFPSSGRGS SKPSLLPKTW TEMDIMEHVG QKLWNHKGRR

201 VLRLRFVCQQ PRGSEVLEFW WHGTSSLDTV FLLLYFNDTQ SVQKTKPLPK

251 GLKEFTEKDP SLLLRRARQA GSIASEVPGP SREHDGPESN QCSLHPFQ**D**S

301 FQQLGWDHWI IAPHLYTPNY CKGVCPRVLH YGLNSPNHAI IQNLVSELVD

351 QNVPQPSCVP YKYVPISILL IEANGSILYK EYEGMIAQSC TCR

Figure 6 The deduced amino acid sequence of ovine GDF-9B (BMP-15) in sheep carrying the Hanna mutation as determined from the nucleotide sequence of ovine GDF-9B (BMP-15) in sheep carrying the Hanna mutation. The position of the premature termination codon is indicated by an asterix

1 MVLLSILRIL LWGLVLFMEH RVQMTQVGQP SIAHLPEAPT LPLIQELLEE

51 APGKQQRKPR VLGHPLRYML ELYQRSADAS GHPRENRTIG ATMVRLVRPL

101 ASVARPLRGS WHIQTLDFPL RPNRVAYQLV RATVVYRHQL HLTHSHLSCH

151 VEPWVQKSPT NHFPSSGRGS SKPSLLPKTW TEMDIMEHVG QKLWNHKGRR

201 VLRLRFVCQQ PRGSEVLEFW WHGTSSLDTV FLLLYFNDTQ SVQKTKPLPK

251 GLKEFTEKDP SLLLRRARQA GSIASEVPGP SREHDGPESN *

Figure 7 The signal sequence polymorphism for GDF-9B in sheep. The predicted signal sequence is 25 amino acids from ATG (Met) to ACA (Thr). The 3 base pair deletion seen in some sheep is one of the two CTT's which are underlined in bold.

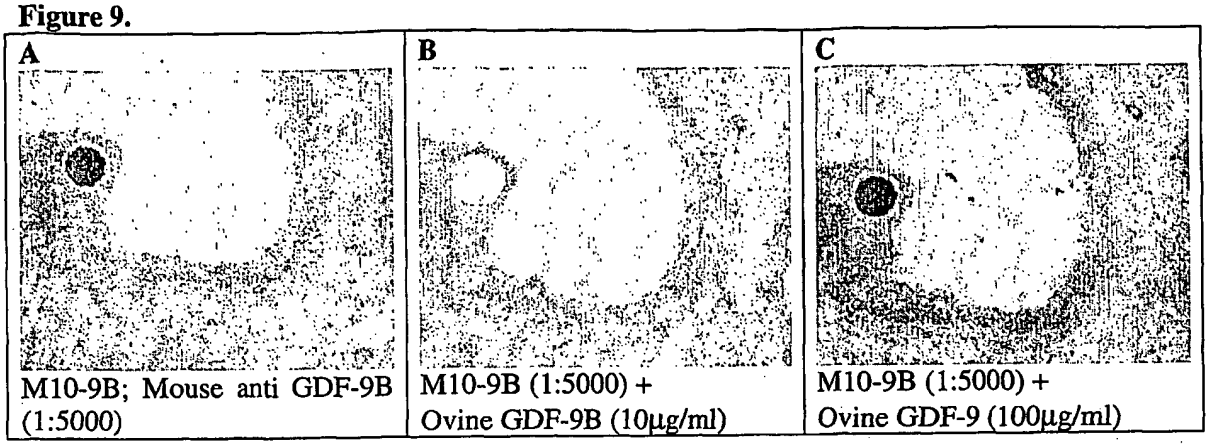
1 ATGGTC CTCCTGAGCA TCCTTAGAAT C**CTTCTT**TGG GGACTGGTGC TTTTTATGGA
ACATAGGGTC
M V L L S I L R I L L W G L V L F M E H R V
CAAATGACA
Q M T

Figure 8 The upstream in-frame atg codon present in sheep genomic DNA. If this codon is used for protein translation initiation the translated protein is illustrated here. Genomic nucleotide sequence is on the upper line and translated amino acid sequence on the lower line. Numbers above the line indicate nucleotide numbers relating to SEQ ID NO: 1, while numbers below the line indicate amino acids relating to Figure 4 The position of the conserved initiation codon is underlined.

```
-27                                                 1
ATG TTG CTG AAC ACC AAG CTT TTC AAG ATG GTC CTC CTG AGC
M  L  L  N  T  K  L  F  K  M  V  L  L  S
                     1

ATC CTT AGA ATC CTT CTT TGG GGA CTG GTG ....
I  L  R  I  L  L  W  G  V  L ....
                     16
```

Figure 9.



Evidence for GDF-9B protein localised to an oocyte. A. Dark staining indicates immunolocalisation of GDF-9B to an oocyte in a sheep ovary. B. Evidence that the staining shown in A can be prevented by preincubation of mouse GDF-9B antibody with an *E. coli* derived mature ovine GDF-9B peptide. C. Evidence that the GDF-9B immunostaining of oocytes can not be removed by preincubation of mouse GDF-9B antibody with an excess of *E. coli* derived mature ovine GDF-9 peptide.

GDF-9BGDF-9B

SEQUENCE LISTING

<110> AgResearch Limited
Davis, George
Galloway, Susan
Juengel, Jenny
Laitinen, Mika
McNatty, Kenneth
Ritvos, Olli
Vuojolainen, Kaisa

<120> Nucleotide and Amino Acid sequences of oocyte factors for altering ovarian follicular growth in vivo or in vitro

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<160> 15 June 2000

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gagatcctac cagaggaaga aacataggac ctgcctgcca gcctttcatt tttccttgcc 120

ctatcctttg tggtagtgga gcctggatgc tgttacccat gtaaaaggaa aggtttaaag 180

cgttatcctt tgggctttta tcagaacatg ttgctgaaca ccaagctttt caag atg 237
Met

gtc ctc ctg agc atc ctt aga atc ctt ctt tgg gga ctg gtg ctt 282
Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val Leu
-265 -260 -255

ttt atg gaa cat agg gtc caa atg aca cag gta ggg cag ccc tct 327
Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro Ser
-250 -245 -240

att gcc cac ctg cct gag gcc cct acc ttg ccc ctg att cag gag 372
Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln Glu
-235 -230 -225

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Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg Val
-220 -215 -210
tta ggg cat ccc tta cgg tat atg ctg gag ctg tac cag cgt tca 462
Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg Ser
-205 -200 -195
gct gac gca agt gga cac cct agg gaa aac cgc acc att ggg gcc 507
Ala Asp Ala Ser Gly His Pro Arg Glu Asn Arg Thr Ile Gly Ala
-190 -185 -180
acc atg gtg agg ctg gtg agg ccg ctg gct agt gta gca agg cct 552
Thr Met Val Arg Leu Val Arg Pro Leu Ala Ser Val Ala Arg Pro
-175 -170 -165
ctc aga g gtgagttatc atactatatt gttctggtgg gagggggga gaaaatgggg 609
Leu Arg
aagaaaagtg tagaaaaaag tggatctgtc agttttctgt caggcttcac attgcctaca 669

gggtaggtgg ttttcaaaag atggcaccct tgggagaacc tggctccaaa tttgcttccc 729

tttagggctc caatttaaga acagattgcc ttggggcctc cctgaggact ttctnagttc 789

tgtatttgag gtgtttttct ccgtctaggg gtatgagtga tctaaaaatg agccacaatt 849

tgtcatctta agggaaaaag acttggactc aaatctttat tctaacaaac actggcttgt 909

gtgtcctctg gcatagcttc tctgagcttc agtttcctcg tctgcaaaat gggaatagca 969

actatctcat aaggctattg tggattcaag agcaaatgca tgtaaagcat ctaatacatt 1029

atataagtgc tcaatagatc gctattatga tcttaaattc atctcaaggc tgcttgtcag 1089

tttgtactga gcaggtctgt tagagaggct aaggctagga tataagaagc taacgctttg 1149

ctcttgttcc ctcttactaa tgcag gc tcc tgg cac ata cag acc ctg gac 1200
Gly Ser Trp His Ile Gln Thr Leu Asp
-160 -155
ttt cct ctg aga cca aac cgg gta gca tac caa cta gtc aga gcc 1245
Phe Pro Leu Arg Pro Asn Arg Val Ala Tyr Gln Leu Val Arg Ala
-150 -145 -140
act gtg gtt tac cgc cat cag ctt cac cta act cat tcc cac ctc 1290
Thr Val Val Tyr Arg His Gln Leu His Leu Thr His Ser His Leu
-135 -130 -125
tcc tgc cat gtg gag ccc tgg gtc cag aaa agc cca acc aat cac 1335

Ser Cys His Val Glu Pro Trp Val Gln Lys Ser Pro Thr Asn His
-120 -115 -110
ttt cct tct tca gga aga ggc tcc tca aag cct tcc ctg ttg ccc aaa 1383
Phe Pro Ser Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu Pro Lys
-105 -100 -95
act tgg aca gag atg gat atc atg gaa cat gtt ggg caa aag ctc tgg 1431
Thr Trp Thr Glu Met Asp Ile Met Glu His Val Gly Gln Lys Leu Trp
-90 -85 -80 -75
aat cac aag ggg cgc agg gtt cta cga ctc cgc ttc gtg tgt cag cag 1479
Asn His Lys Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys Gln Gln
-70 -65 -60
cca aga ggt agt gag gtt ctt gag ttc tgg tgg cat ggc act tca tca 1527
Pro Arg Gly Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser
-55 -50 -45
ttg gac act gtc ttc ttg tta ctg tat ttc aat gac act cag agt gtt 1575
Leu Asp Thr Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr Gln Ser Val
-40 -35 -30
cag aag acc aaa cct ctc cct aaa ggc ctg aaa gag ttt aca gaa aaa 1623
Gln Lys Thr Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys
-25 -20 -15
gac cct tct ctt ctc ttg agg agg gct cgt caa gca ggc agt att gca 1671
Asp Pro Ser Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala
-10 -5 -1 1 5
tcg gaa gtt cct ggc ccc tcc agg gag cat gat ggg cct gaa agt aac 1719
Ser Glu Val Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn
10 15 20
cag tgt tcc ctc cac cct ttt caa gtc agc ttc cag cag ctg ggc tgg 1767
Gln Cys Ser Leu His Pro Phe Gln Val Ser Phe Gln Gln Leu Gly Trp
25 30 35
gat cac tgg atc att gct ccc cat ctc tat acc cca aac tac tgt aag 1815
Asp His Trp Ile Ile Ala Pro His Leu Tyr Thr Pro Asn Tyr Cys Lys
40 45 50
gga gta tgt cct cgg gta cta cac tat ggt ctc aat tct ccc aat cat 1863
Gly Val Cys Pro Arg Val Leu His Tyr Gly Leu Asn Ser Pro Asn His
55 60 65 70
gcc atc atc cag aac ctt gtc agt gag ctg gtg gat cag aat gtc cct 1911
Ala Ile Ile Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro
75 80 85
cag cct tcc tgt gtc cct tat aag tat gtt ccc att agc atc ctt ctg 1959
Gln Pro Ser Cys Val Pro Tyr Lys Tyr Val Pro Ile Ser Ile Leu Leu
90 95 100

att gag gca aat ggg agt atc ttg tac aag gag tat gag ggt atg att 2007
Ile Glu Ala Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile
105 110 115
gcc cag tcc tgc aca tgc agg tgacggcaaa ggtgca 2044
Ala Gln Ser Cys Thr Cys Arg
120 125

<210> 2
<211> 393
<212> PRT
<213> Ovis aries
<220>
<221> misc_feature
<222> (235)..(237)
<223> atg start codon
<220>
<221> misc_feature
<222> (208)..(210)
<223> in frame 5' atg codon
<220>
<221> misc_feature
<222> (784)..()
<223> n represents approximately 5.4 kb of unsequenced intron
<220>
<221> misc_feature
<222> (2029)..(2031)
<223> tga stop codon
<400> 2

Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val
-265 -260 -255
Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro
-250 -245 -240
Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
-235 -230 -225
Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg
-220 -215 -210
Val Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg
-205 -200 -195
Ser Ala Asp Ala Ser Gly His Pro Arg Glu Asn Arg Thr Ile Gly
-190 -185 -180

Ala Thr Met Val Arg Leu Val Arg Pro Leu Ala Ser Val Ala Arg
-175 -170 -165
Pro Leu Arg Gly Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu
-160 -155 -150
Arg Pro Asn Arg Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val
-145 -140 -135
Tyr Arg His Gln Leu His Leu Thr His Ser His Leu Ser Cys His
-130 -125 -120
Val Glu Pro Trp Val Gln Lys Ser Pro Thr Asn His Phe Pro Ser
-115 -110 -105
Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr
-100 -95 -90
Glu Met Asp Ile Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys
-85 -80 -75
Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly
-70 -65 -60
Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr
-55 -50 -45 -40
Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr
-35 -30 -25
Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser
-20 -15 -10
Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val
-5 -1 1 5
Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser
10 15 20 25
Leu His Pro Phe Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp
30 35 40
Ile Ile Ala Pro His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys
45 50 55
Pro Arg Val Leu His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile
60 65 70
Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser
75 80 85
Cys Val Pro Tyr Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala
90 95 100 105
Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser
110 115 120
Cys Thr Cys Arg
125

<210> 3
<211> 1195
<212> DNA
<213> Ovis aries
<220>
<221> CDS
<222> (1)..(1179)
<220>
<221> misc_feature
<222> (325)..(326)
<223> position of intron in genomic sequence
<220>
<221> misc_feature
<222> (793)..(804)
<223> furin protease cleavage sequence
<220>
<221> mat_peptide
<222> (805)..()
<220>
<221> misc_feature
<222> (896)..()
<223> positon of Inverdale mutation
<400> 3
atg gtc ctc ctg agc atc ctt aga atc ctt ctt tgg gga ctg gtg 45
Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val
-265 -260 -255
ctt ttt atg gaa cat agg gtc caa atg aca cag gta ggg cag ccc 90
Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro
-250 -245 -240
tct att gcc cac ctg cct gag gcc cct acc ttg ccc ctg att cag 135
Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
-235 -230 -225
gag ctg cta gaa gaa gcc cct ggc aag cag cag agg aag ccg cgg 180
Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg
-220 -215 -210
gtc tta ggg cat ccc tta cgg tat atg ctg gag ctg tac cag cgt 225
Val Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg
-205 -200 -195
tca gct gac gca agt gga cac cct agg gaa aac cgc acc att ggg 270
Ser Ala Asp Ala Ser Gly His Pro Arg Glu Asn Arg Thr Ile Gly
-190 -185 -180

gcc acc atg gtg agg ctg gtg agg ccg ctg gct agt gta gca agg 315
Ala Thr Met Val Arg Leu Val Arg Pro Leu Ala Ser Val Ala Arg
-175 -170 -165
cct ctc aga ggc tcc tgg cac ata cag acc ctg gac ttt cct ctg 360
Pro Leu Arg Gly Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu
-160 -155 -150
aga cca aac cgg gta gca tac caa cta gtc aga gcc act gtg gtt 405
Arg Pro Asn Arg Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val
-145 -140 -135
tac cgc cat cag ctt cac cta act cat tcc cac ctc tcc tgc cat 450
Tyr Arg His Gln Leu His Leu Thr His Ser His Leu Ser Cys His
-130 -125 -120
gtg gag ccc tgg gtc cag aaa agc cca acc aat cac ttt cct tct 495
Val Glu Pro Trp Val Gln Lys Ser Pro Thr Asn His Phe Pro Ser
-115 -110 -105
tca gga aga ggc tcc tca aag cct tcc ctg ttg ccc aaa act tgg aca 543
Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr
-100 -95 -90
gag atg gat atc atg gaa cat gtt ggg caa aag ctc tgg aat cac aag 591
Glu Met Asp Ile Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys
-85 -80 -75
ggg cgc agg gtt cta cga ctc cgc ttc gtg tgt cag cag cca aga ggt 639
Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly
-70 -65 -60
agt gag gtt ctt gag ttc tgg tgg cat ggc act tca tca ttg gac act 687
Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr
-55 -50 -45 -40
gtc ttc ttg tta ctg tat ttc aat gac act cag agt gtt cag aag acc 735
Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr
-35 -30 -25
aaa cct ctc cct aaa ggc ctg aaa gag ttt aca gaa aaa gac cct tct 783
Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser
-20 -15 -10
ctt ctc ttg agg agg gct cgt caa gca ggc agt att gca tcg gaa gtt 831
Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val
-5 -1 1 5
cct ggc ccc tcc agg gag cat gat ggg cct gaa agt aac cag tgt tcc 879
Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser
10 15 20 25
ctc cac cct ttt caa gac agc ttc cag cag ctg ggc tgg gat cac tgg 927
Leu His Pro Phe Gln Asp Ser Phe Gln Gln Leu Gly Trp Asp His Trp

30 35 40
atc att gct ccc cat ctc tat acc cca aac tac tgt aag gga gta tgt 975
Ile Ile Ala Pro His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys
45 50 55
cct cgg gta cta cac tat ggt ctc aat tct ccc aat cat gcc atc atc 1023
Pro Arg Val Leu His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile
60 65 70
cag aac ctt gtc agt gag ctg gtg gat cag aat gtc cct cag cct tcc 1071
Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser
75 80 85
tgt gtc cct tat aag tat gtt ccc att agc atc ctt ctg att gag gca 1119
Cys Val Pro Tyr Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala
90 95 100 105
aat ggg agt atc ttg tac aag gag tat gag ggt atg att gcc cag tcc 1167
Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser
110 115 120
tgc aca tgc agg tgacggcaaa ggtgca 1195
Cys Thr Cys Arg
125

<210> 4
<211> 393
<212> PRT
<213> Ovis aries
<220>
<221> misc_feature
<222> (325)..(326)
<223> position of intron in genomic sequence
<220>
<221> misc_feature
<222> (793)..(804)
<223> furin protease cleavage sequence
<220>
<221> misc_feature
<222> (896)..()
<223> positon of Inverdale mutation
<400> 4
Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val
-265 -260 -255
Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro
-250 -245 -240

Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
-235 -230 -225
Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg
-220 -215 -210
Val Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg
-205 -200 -195
Ser Ala Asp Ala Ser Gly His Pro Arg Glu Asn Arg Thr Ile Gly
-190 -185 -180
Ala Thr Met Val Arg Leu Val Arg Pro Leu Ala Ser Val Ala Arg
-175 -170 -165
Pro Leu Arg Gly Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu
-160 -155 -150
Arg Pro Asn Arg Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val
-145 -140 -135
Tyr Arg His Gln Leu His Leu Thr His Ser His Leu Ser Cys His
-130 -125 -120
Val Glu Pro Trp Val Gln Lys Ser Pro Thr Asn His Phe Pro Ser
-115 -110 -105
Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr
-100 -95 -90
Glu Met Asp Ile Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys
-85 -80 -75
Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly
-70 -65 -60
Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr
-55 -50 -45 -40
Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr
-35 -30 -25
Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser
-20 -15 -10
Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val
-5 -1 1 5
Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser
10 15 20 25
Leu His Pro Phe Gln Asp Ser Phe Gln Gln Leu Gly Trp Asp His Trp
30 35 40
Ile Ile Ala Pro His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys
45 50 55
Pro Arg Val Leu His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile
60 65 70
Gln Asn Leu Val Ser Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser

75 80 85
Cys Val Pro Tyr Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala
90 95 100 105
Asn Gly Ser Ile Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser
110 115 120
Cys Thr Cys Arg
125

<210> 5
<211> 1195
<212> DNA
<213> Ovis aries
<220>
<221> CDS
<222> (1)..(870)
<220>
<221> misc_feature
<222> (325)..(326)
<223> position of intron in genomic sequence
<220>
<221> misc_feature
<222> (793)..(804)
<223> furin protease cleavage sequence
<220>
<221> mat_peptide
<222> (805)..()
<220>
<221> misc_feature
<222> (871)..()
<223> position of Hanna mutation
<400> 5
atg gtc ctc ctg agc atc ctt aga atc ctt ctt tgg gga ctg gtg 45
Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val
-265 -260 -255
ctt ttt atg gaa cat agg gtc caa atg aca cag gta ggg cag ccc 90
Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro
-250 -245 -240
tct att gcc cac ctg cct gag gcc cct acc ttg ccc ctg att cag 135
Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
-235 -230 -225
gag ctg cta gaa gaa gcc cct ggc aag cag cag agg aag ccg cgg 180
Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg

-220 -215 -210

gtc tta ggg cat ccc tta cgg tat atg ctg gag ctg tac cag cgt 225

Val Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg

-205 -200 -195

tca gct gac gca agt gga cac cct agg gaa aac cgc acc att ggg 270

Ser Ala Asp Ala Ser Gly His Pro Arg Glu Asn Arg Thr Ile Gly

-190 -185 -180

gcc acc atg gtg agg ctg gtg agg ccg ctg gct agt gta gca agg 315

Ala Thr Met Val Arg Leu Val Arg Pro Leu Ala Ser Val Ala Arg

-175 -170 -165

cct ctc aga ggc tcc tgg cac ata cag acc ctg gac ttt cct ctg 360

Pro Leu Arg Gly Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu

-160 -155 -150

aga cca aac cgg gta gca tac caa cta gtc aga gcc act gtg gtt 405

Arg Pro Asn Arg Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val

-145 -140 -135

tac cgc cat cag ctt cac cta act cat tcc cac ctc tcc tgc cat 450

Tyr Arg His Gln Leu His Leu Thr His Ser His Leu Ser Cys His

-130 -125 -120

gtg gag ccc tgg gtc cag aaa agc cca acc aat cac ttt cct tct 495

Val Glu Pro Trp Val Gln Lys Ser Pro Thr Asn His Phe Pro Ser

-115 -110 -105

tca gga aga ggc tcc tca aag cct tcc ctg ttg ccc aaa act tgg aca 543

Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr

-100 -95 -90

gag atg gat atc atg gaa cat gtt ggg caa aag ctc tgg aat cac aag 591

Glu Met Asp Ile Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys

-85 -80 -75

ggg cgc agg gtt cta cga ctc cgc ttc gtg tgt cag cag cca aga ggt 639

Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly

-70 -65 -60

agt gag gtt ctt gag ttc tgg tgg cat ggc act tca tca ttg gac act 687

Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr

-55 -50 -45 -40

gtc ttc ttg tta ctg tat ttc aat gac act cag agt gtt cag aag acc 735

Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr

-35 -30 -25

aaa cct ctc cct aaa ggc ctg aaa gag ttt aca gaa aaa gac cct tct 783

Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser

-20 -15 -10

ctt ctc ttg agg agg gct cgt caa gca ggc agt att gca tcg gaa gtt 831

Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val
-5 -1 1 5

cct ggc ccc tcc agg gag cat gat ggg cct gaa agt aac tagtgttccc 880
Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn
10 15 20

tccacccttt tcaagtcagc ttccagcagc tgggctggga tcactggatc attgctcccc 940
atctctatac cccaaactac tgtaagggag tatgtcctcg ggtactacac tatggtctca 1000
attctcccaa tcatgccatc atccagaacc ttgtcagtga gctggtggat cagaatgtcc 1060
ctcagccttc ctgtgtccct tataagtatg ttcccattag catccttctg attgaggcaa 1120
atgggagtat cttgtacaag gagtatgagg gtatgattgc ccagtcctgc acatgcaggt 1180
gacggcaaag gtgca 1195

<210> 6
<211> 290
<212> PRT
<213> Ovis aries
<220>
<221> misc_feature
<222> (325)..(326)
<223> position of intron in genomic sequence
<220>
<221> misc_feature
<222> (793)..(804)
<223> furin protease cleavage sequence
<220>
<221> misc_feature
<222> (871)..()
<223> position of Hanna mutation
<400> 6

Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val
-265 -260 -255

Leu Phe Met Glu His Arg Val Gln Met Thr Gln Val Gly Gln Pro
-250 -245 -240

Ser Ile Ala His Leu Pro Glu Ala Pro Thr Leu Pro Leu Ile Gln
-235 -230 -225

Glu Leu Leu Glu Glu Ala Pro Gly Lys Gln Gln Arg Lys Pro Arg
-220 -215 -210

Val Leu Gly His Pro Leu Arg Tyr Met Leu Glu Leu Tyr Gln Arg
-205 -200 -195

Ser Ala Asp Ala Ser Gly His Pro Arg Glu Asn Arg Thr Ile Gly
-190 -185 -180

Ala Thr Met Val Arg Leu Val Arg Pro Leu Ala Ser Val Ala Arg

-175 -170 -165
Pro Leu Arg Gly Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu
-160 -155 -150
Arg Pro Asn Arg Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val
-145 -140 -135
Tyr Arg His Gln Leu His Leu Thr His Ser His Leu Ser Cys His
-130 -125 -120
Val Glu Pro Trp Val Gln Lys Ser Pro Thr Asn His Phe Pro Ser
-115 -110 -105
Ser Gly Arg Gly Ser Ser Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr
-100 -95 -90
Glu Met Asp Ile Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys
-85 -80 -75
Gly Arg Arg Val Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly
-70 -65 -60
Ser Glu Val Leu Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr
-55 -50 -45 -40
Val Phe Leu Leu Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr
-35 -30 -25
Lys Pro Leu Pro Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser
-20 -15 -10
Leu Leu Leu Arg Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val
-5 -1 1 5
Pro Gly Pro Ser Arg Glu His Asp Gly Pro Glu Ser Asn
10 15 20

<210> 7
<211> 75
<212> DNA
<213> Ovis aries
<220>
<221> CDS
<222> (1)..(75)
<220>
<221> misc_feature
<222> (28)..(30)
<223> ctt codon which is deleted in some sheep
<400> 7
atg gtc ctc ctg agc atc ctt aga atc ctt ctt tgg gga ctg gtg ctt 48
Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val Leu
1 5 10 15

ttt atg gaa cat agg gtc caa atg aca 75
Phe Met Glu His Arg Val Gln Met Thr
20 25

<210> 8
<211> 25
<212> PRT
<213> Ovis aries
<220>
<221> misc_feature
<222> (28)..(30)
<223> ctt codon which is deleted in some sheep
<400> 8
Met Val Leu Leu Ser Ile Leu Arg Ile Leu Leu Trp Gly Leu Val Leu
1 5 10 15
Phe Met Glu His Arg Val Gln Met Thr
20 25

<210> 9
<211> 72
<212> DNA
<213> Ovis aries
<220>
<221> misc_feature
<222> (28)..(30)
<223> Probable atg start codon
<220>
<221> CDS
<222> (1)..(72)
<223> cds if 3' atg is used
<220>
<221> misc_feature
<222> (1)..(72)
<223> cds if 3' atg start codon is used
<400> 9
atg ttg ctg aac acc aag ctt ttc aag atg gtc ctc ctg agc atc ctt 48
Met Leu Leu Asn Thr Lys Leu Phe Lys Met Val Leu Leu Ser Ile Leu
1 5 10 15
aga atc ctt ctt tgg gga ctg gtg 72
Arg Ile Leu Leu Trp Gly Leu Val
20

<210> 10

<211> 24
<212> PRT
<213> Ovis aries
<220>
<221> misc_feature
<222> (28)..(30)
<223> Probable atg start codon
<220>
<221> misc_feature
<222> (1)..(72)
<223> cds if 3' atg start codon is used
<400> 10
Met Leu Leu Asn Thr Lys Leu Phe Lys Met Val Leu Leu Ser Ile Leu
1 5 10 15
Arg Ile Leu Leu Trp Gly Leu Val
20
<210> 11
<211> 759
<212> DNA
<213> Sus scrofa
<220>
<221> CDS
<222> (2)..(757)
<223> sequence stops 5 nucleotides short of the stop codon
<220>
<221> mat_peptide
<222> (389)..()
<400> 11
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-125 -120 -115
atc cag aaa agc aca acc agt cac ttt cct tcc tca gga aga ggc 91
Ile Gln Lys Ser Thr Thr Ser His Phe Pro Ser Ser Gly Arg Gly
-110 -105 -100
tcc tta aag cct tcc ctg ctg ccc caa gct tgg acg gag atg gat gtc 139
Ser Leu Lys Pro Ser Leu Leu Pro Gln Ala Trp Thr Glu Met Asp Val
-95 -90 -85
acg caa cat gtt gga caa aag ctc tgg aat cac aag ggg cgc agg gtt 187
Thr Gln His Val Gly Gln Lys Leu Trp Asn His Lys Gly Arg Arg Val
-80 -75 -70
cta cga ctc cgc ttc atg tgt cag cag caa aat ggt agt gag att ctt 235

Leu Arg Leu Arg Phe Met Cys Gln Gln Gln Asn Gly Ser Glu Ile Leu
-65 -60 -55
gag ttc cgg ggg cgt ggc att tca tcc ctg gac act gcc ttc ttg tta 283
Glu Phe Arg Gly Arg Gly Ile Ser Ser Leu Asp Thr Ala Phe Leu Leu
-50 -45 -40
ctc tat ttc aat gac act cgg agt gtt cag aag gcc aaa ctt ctt ccc 331
Leu Tyr Phe Asn Asp Thr Arg Ser Val Gln Lys Ala Lys Leu Leu Pro
-35 -30 -25 -20
aga ggc ctg gaa gag ttt atg gca aga gac cct tct ctt ctt ttg cgg 379
Arg Gly Leu Glu Glu Phe Met Ala Arg Asp Pro Ser Leu Leu Leu Arg
-15 -10 -5
aag gcc cgg caa gca ggc agc atc gca tct gag gtt ctt ggc ccc tcc 427
Lys Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Leu Gly Pro Ser
-1 1 5 10
agg gag cac gat ggg cct gaa agt aac cag tgt tcc ctc cat cct ttc 475
Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe
15 20 25
caa gtc agc ttc cac caa ctg ggt tgg gat cat tgg atc att gct ccc 523
Gln Val Ser Phe His Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro
30 35 40 45
cat ttc tat acc cca aac tac tgt aag ggg gtc tgc cct cgg gta cta 571
His Phe Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu
50 55 60
cac tat ggt ctc aat tcc ccc aat cat gcc atc atc cag aac ctt gtc 619
His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val
65 70 75
aat gag ctg gtg gac cag agt gtc cct cag ccc tcc tgt gtc cct tat 667
Asn Glu Leu Val Asp Gln Ser Val Pro Gln Pro Ser Cys Val Pro Tyr
80 85 90
aag tat gtg cct att agc atc ctc ctg att gag gca aat ggg agt atc 715
Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile
95 100 105
ttg tac aag gag tat gag gat atg att gcc cag tcc tgt acg tg 759
Leu Tyr Lys Glu Tyr Glu Asp Met Ile Ala Gln Ser Cys Thr
110 115 120

<210> 12
<211> 252
<212> PRT
<213> Sus scrofa
<400> 12

Leu His Leu Ala Pro Phe His Leu Ser Cys His Val Glu Pro Trp
-125 -120 -115
Ile Gln Lys Ser Thr Thr Ser His Phe Pro Ser Ser Gly Arg Gly
-110 -105 -100
Ser Leu Lys Pro Ser Leu Leu Pro Gln Ala Trp Thr Glu Met Asp Val
-95 -90 -85
Thr Gln His Val Gly Gln Lys Leu Trp Asn His Lys Gly Arg Arg Val
-80 -75 -70
Leu Arg Leu Arg Phe Met Cys Gln Gln Gln Asn Gly Ser Glu Ile Leu
-65 -60 -55
Glu Phe Arg Gly Arg Gly Ile Ser Ser Leu Asp Thr Ala Phe Leu Leu
-50 -45 -40
Leu Tyr Phe Asn Asp Thr Arg Ser Val Gln Lys Ala Lys Leu Leu Pro
-35 -30 -25 -20
Arg Gly Leu Glu Glu Phe Met Ala Arg Asp Pro Ser Leu Leu Leu Arg
-15 -10 -5
Lys Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Leu Gly Pro Ser
-1 1 5 10
Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe
15 20 25
Gln Val Ser Phe His Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro
30 35 40 45
His Phe Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu
50 55 60
His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val
65 70 75
Asn Glu Leu Val Asp Gln Ser Val Pro Gln Pro Ser Cys Val Pro Tyr
80 85 90
Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile
95 100 105
Leu Tyr Lys Glu Tyr Glu Asp Met Ile Ala Gln Ser Cys Thr
110 115 120

<210> 13
<211> 857
<212> DNA
<213> Cervus elaphus
<220>
<221> CDS
<222> (3)..(854)
<220>

<221> mat_peptide
<222> (480)..()
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Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg
-155 -150 -145
gta gcc tac caa cta gtc aga gcc act gtg gtt tac cgc cat caa 92
Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val Tyr Arg His Gln
-140 -135 -130
ctt cac cta act cat tcc cac ctc tcc tgc cat gtg gag ccc tgg 137
Leu His Leu Thr His Ser His Leu Ser Cys His Val Glu Pro Trp
-125 -120 -115
atc cag aaa agc cca acc agt cac ttt cct tct tca gga aga ggc 182
Ile Gln Lys Ser Pro Thr Ser His Phe Pro Ser Ser Gly Arg Gly
-110 -105 -100
tcc tca aag cct tcc ctg ctg ccc aaa gct tgg aca gag atg gat atc 230
Ser Ser Lys Pro Ser Leu Leu Pro Lys Ala Trp Thr Glu Met Asp Ile
-95 -90 -85
atg gaa cat gtt gga caa aag ctg tgg aat cgc aag ggg cgc agg gtt 278
Met Glu His Val Gly Gln Lys Leu Trp Asn Arg Lys Gly Arg Arg Val
-80 -75 -70
cta cga ctc cgc ttc atg tgt cag cag cca aga ggt agt gag gtt ctt 326
Leu Arg Leu Arg Phe Met Cys Gln Gln Pro Arg Gly Ser Glu Val Leu
-65 -60 -55
gag ttc tgg tgg cat ggc act tca tca ttg gac act gtc ttc ttg tta 374
Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr Val Phe Leu Leu
-50 -45 -40
ctg tat ttc aat gac act cag agt gtt cag aag acc aaa cct ctc cct 422
Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr Lys Pro Leu Pro
-35 -30 -25 -20
aaa ggc ctg aaa gag ttt aca gaa aaa gac cct tct ctt ctc ttg agg 470
Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser Leu Leu Leu Arg
-15 -10 -5
agg gct cgt caa gca ggc agt atc gca tct gaa gtt cct ggc ccc tcc 518
Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Pro Gly Pro Ser
-1 1 5 10
agg gag cat gat ggg cct gaa agt aac cag tgt tcc ctc cac cct ttt 566
Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe
15 20 25
caa gtc agc ttc cag cag ctg ggc tgg gat cac tgg atc att gct ccc 614
Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro

30 35 40 45

aat ctc tat acc cca aac tac tgt aag gga gtg tgt cct cgg gta cta 662
Asn Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu
50 55 60

cac tat ggt ctc aat tct ccc aat cat gcc atc atc cag aac ctt gtc 710
His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val
65 70 75

aat gag ctg gtg gat cag agt gtc cct cag cct tcc tgt gtc cct tat 758
Asn Glu Leu Val Asp Gln Ser Val Pro Gln Pro Ser Cys Val Pro Tyr
80 85 90

aag tat gtt ccc att agc atc ctg ctg att gag gca aat ggg agt atc 806
Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile
95 100 105

ttg tac aag gag tat gag ggt atg att gcc cag tcc tgc aca tgc agg 854
Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser Cys Thr Cys Arg
110 115 120 125

tga 857

<210> 14
<211> 284
<212> PRT
<213> Cervus elaphus
<400> 14

Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg
-155 -150 -145
Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val Tyr Arg His Gln
-140 -135 -130
Leu His Leu Thr His Ser His Leu Ser Cys His Val Glu Pro Trp
-125 -120 -115
Ile Gln Lys Ser Pro Thr Ser His Phe Pro Ser Ser Gly Arg Gly
-110 -105 -100
Ser Ser Lys Pro Ser Leu Leu Pro Lys Ala Trp Thr Glu Met Asp Ile
-95 -90 -85
Met Glu His Val Gly Gln Lys Leu Trp Asn Arg Lys Gly Arg Arg Val
-80 -75 -70
Leu Arg Leu Arg Phe Met Cys Gln Gln Pro Arg Gly Ser Glu Val Leu
-65 -60 -55
Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr Val Phe Leu Leu
-50 -45 -40
Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr Lys Pro Leu Pro
-35 -30 -25 -20
Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser Leu Leu Leu Arg

-15 -10 -5
Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Pro Gly Pro Ser
-1 1 5 10
Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe
15 20 25
Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro
30 35 40 45
Asn Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu
50 55 60
His Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val
65 70 75
Asn Glu Leu Val Asp Gln Ser Val Pro Gln Pro Ser Cys Val Pro Tyr
80 85 90
Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile
95 100 105
Leu Tyr Lys Glu Tyr Glu Gly Met Ile Ala Gln Ser Cys Thr Cys Arg
110 115 120 125

<210> 15
<211> 857
<212> DNA
<213> Capra hircus
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<221> CDS
<222> (3)..(854)
<220>
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<222> (480)..()
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Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg
-155 -150 -145

gta gca tac caa cta gtc aga gcc act gtg gtt tac cgc cat cag 92
Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val Tyr Arg His Gln
-140 -135 -130

ctt cac cta act cat tcc cac ctc tcc tgc cat gtg gag ccc tgg 137
Leu His Leu Thr His Ser His Leu Ser Cys His Val Glu Pro Trp
-125 -120 -115

ggg cag aaa agc cca acc aat cac ttt cct tct tca gga aga ggc 182
Gly Gln Lys Ser Pro Thr Asn His Phe Pro Ser Ser Gly Arg Gly
-110 -105 -100

tcc cca aag cct tcc ctg ttg ccc aaa act tgg aca gag atg gat atc 230

Ser Pro Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr Glu Met Asp Ile
-95 -90 -85
atg gaa cat gtt ggg caa aag ctc tgg aat cac aag ggg cgc agg gtt 278
Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys Gly Arg Arg Val
-80 -75 -70
cta cga ctc cgc ttc gta tgt cag cag cca aga ggt agt gag gtt ctt 326
Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly Ser Glu Val Leu
-65 -60 -55
gag ttc tgg tgg cat ggc act tca tca ttg gac act gtc ttc ttg tta 374
Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr Val Phe Leu Leu
-50 -45 -40
ctg tat ttc aat gac act cag agt gtt cag aaa acc aaa cct ctc cct 422
Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr Lys Pro Leu Pro
-35 -30 -25 -20
aaa ggc ctg aaa gag ttt aca gaa aaa gac cct tct ctt ctc ttg agg 470
Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser Leu Leu Leu Arg
-15 -10 -5
agg gct cgt caa gca ggc agt att gca tct gaa gtt cct ggc ccc tcc 518
Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Pro Gly Pro Ser
-1 1 5 10
agg gag cat gat ggg cct gaa agt aac cag tgt tcc ctc cac cct ttt 566
Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe
15 20 25
caa gtc agc ttc cag cag ctg ggc tgg gat cac tgg atc att gct ccc 614
Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro
30 35 40 45
cat ctc tat acc cca aac tac tgt aag gga gta tgt cct cgg gta cta 662
His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu
50 55 60
tac tat ggt ctc aat tct ccc aat cat gcc atc atc cag aac ctt gtc 710
Tyr Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val
65 70 75
aat gag ctg gtg gat cag aat gtc cct cag cct tcc tgt gtc cct tat 758
Asn Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser Cys Val Pro Tyr
80 85 90
aag tat gtt ccc att agc atc ctt ctg att gag gca aat ggg agt atc 806
Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile
95 100 105
ttg tac aag gag tat gag ggt atg att gcc cag tcc tgc aca tgc agg 854
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110 115 120 125

tga 857

<210> 16

<211> 284

<212> PRT

<213> Capra hircus

<400> 16

Ser Trp His Ile Gln Thr Leu Asp Phe Pro Leu Arg Pro Asn Arg
-155 -150 -145

Val Ala Tyr Gln Leu Val Arg Ala Thr Val Val Tyr Arg His Gln
-140 -135 -130

Leu His Leu Thr His Ser His Leu Ser Cys His Val Glu Pro Trp
-125 -120 -115

Gly Gln Lys Ser Pro Thr Asn His Phe Pro Ser Ser Gly Arg Gly
-110 -105 -100

Ser Pro Lys Pro Ser Leu Leu Pro Lys Thr Trp Thr Glu Met Asp Ile
-95 -90 -85

Met Glu His Val Gly Gln Lys Leu Trp Asn His Lys Gly Arg Arg Val
-80 -75 -70

Leu Arg Leu Arg Phe Val Cys Gln Gln Pro Arg Gly Ser Glu Val Leu
-65 -60 -55

Glu Phe Trp Trp His Gly Thr Ser Ser Leu Asp Thr Val Phe Leu Leu
-50 -45 -40

Leu Tyr Phe Asn Asp Thr Gln Ser Val Gln Lys Thr Lys Pro Leu Pro
-35 -30 -25 -20

Lys Gly Leu Lys Glu Phe Thr Glu Lys Asp Pro Ser Leu Leu Leu Arg
-15 -10 -5

Arg Ala Arg Gln Ala Gly Ser Ile Ala Ser Glu Val Pro Gly Pro Ser
-1 1 5 10

Arg Glu His Asp Gly Pro Glu Ser Asn Gln Cys Ser Leu His Pro Phe
15 20 25

Gln Val Ser Phe Gln Gln Leu Gly Trp Asp His Trp Ile Ile Ala Pro
30 35 40 45

His Leu Tyr Thr Pro Asn Tyr Cys Lys Gly Val Cys Pro Arg Val Leu
50 55 60

Tyr Tyr Gly Leu Asn Ser Pro Asn His Ala Ile Ile Gln Asn Leu Val
65 70 75

Asn Glu Leu Val Asp Gln Asn Val Pro Gln Pro Ser Cys Val Pro Tyr
80 85 90

Lys Tyr Val Pro Ile Ser Ile Leu Leu Ile Glu Ala Asn Gly Ser Ile
95 100 105